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O S T E O P O R O S I S

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TABLE OF CONTENTS

Volume I

TABLE OF CONTENTS

Volume II

PREFACE

ACKNOWLEDGEMENTS

My interest in calcium metabolism dates from the taking up of an M.R.C. supported Research Fellowship in 1961 with Professor B.E.C. Nordin, then Senior Lecturer in the Department of Medicine in the Western Infirmary, Glasgow, and now Director of the M.R.C. Mineral Metabolism Research Unit in The General Infirmary, Leeds. No one who has known Professor Nordin can fail to be affected by his enormous enthusiasm and drive, and I am grateful for his ready help and encouragement at all times.

I wish also to acknowledge the help and constructive criticism of Professor Sir Edward Wayne, and Professor G.M. Wilson, and I am grateful to both of them for the use of generous laboratory facilities.

A large number of different types of investigations have contributed to this study on osteoporosis, and I should like to acknowledge the assistance of the collaborators named in the list of publications at the end of the text in Volume I. The data for the incidence of fracture in the normal population, which has been related to the changes in bone mass, is taken from the study of Knowelden, Buhr and Dunbar (1964).

I would in particular like to thank Mr. J. Shimmins for help in the isotope studies, and Mr. J. Anderson for taking the X-rays of the patients studied.

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INTRODUCTION

I N T R O D U C T I O N

The investigations described in this thesis are concerned largely, but not exclusively, with the study of primary osteoporosis. This is a condition now known to affect a large proportion of the population, especially elderly women, but in which the aetiological factors are poorly understood. Some aspects of osteoporosis secondary to known predisposing aetiological factors have also been considered where they help to throw light on the problems of primary osteoporosis.

The problems studied and the present position of knowledge relating to each is outlined below, and examined in more detail at the beginning of each Chapter. The extent to which answers have been obtained to each of the problems posed have been examined at the end of the thesis under the general heading 'Conclusions', and under the same sub-headings which introduce the problems below.

Since a large number of Tables and Figures are presented, the thesis has been divided into two volumes, the first comprising of the text and the other of the Tables, Illustrations, and Appendices.

1) The problems of definition

Osteoporosis has been known to play an important role in the problems of advancing age for many years, but it is not a condition which can be laid solely at the doorstep of advancing mechanisation and modern diet. Fyfe (1958) reported osteoporosis affecting the human skeleton from pre-historic times. In 1885, Pommer was the first to distinguish between the histological appearances of osteoporosis and osteomalacia, but the clinical entities continued to be confused for some time thereafter. Osteoporosis was not in fact recognised as a clinical entity until so described by Albright, Burnett, Cope and Parsons in 1941. Since that time a great deal of attention has been directed to the investigation of this condition, but nearly 30 years later, no clear quantitative definition of osteoporosis exists.

The difficulties associated with definition are all the more surprising in view of the quantitative measurements which have been made with increasing frequency over the last decade. The problems of definition arise for a number of reasons. The definition of any condition which causes disease is partly dependent upon the consideration of aetiological

factors. In the case of osteoporosis, these are poorly understood. It also partly depends upon the development of methods which accurately measure the effects of the condition on the bone. Definition is also concerned with clinical manifestations which, in osteoporosis, are not exclusive to the condition.

The qualitative definition of osteoporosis is that it is a condition in which the amount of bone present is diminished, but the bone remaining is biochemically normal. Even here some modification may be necessary for the sake of precision as some loss of mineral without loss of matrix has been reported by Jowsey and Gershon-Cohen (1964) and Dollerup (1964). However, even ignoring this point, examination of this definition immediately leads to the discovery of difficulties in quantitative definition. Clearly, the definition requires the definition of normal limits and though normal surveys have been carried out, little use has been made of these for clinical purposes. The definition of normal limits does not necessarily help, since any subject at the top of the normal scale may lose a significant amount of bone without reaching the lower normal limit, yet from the dynamic point of view, the subject having lost bone should be considered to have developed a degree of osteoporosis.

In other words, the setting of a lower limit of normality could be quite artificial and unhelpful when considering development of the pathological state.

A further problem arises from the fact that the skeletal mass is changing throughout life. Clearly the skeleton continues to grow until the epiphyses close in the young adult after which no further growth in length has been reported to occur. However, recent reports have described continued growth in the external diameter of the metacarpals (Garn, Rohmann, Wagner and Ascoli, 1967), the femur (Smith and Rizek, 1966), and the rib (Epker and Frost, 1966). At the same time the internal diameter continues to increase throughout life (Epker and Frost, 1965). In addition, both the cortical and trabecular bones show changes in density, number and thickness of trabeculae, and in porosity of the cortical bone with time (Rowland, Jowsey and Marshall, 1958; Jowsey, 1963a and 1963b; Epker and Frost, 1965).

Further problems have arisen because it is not always clear in the literature as to the nature of the physical measurements made. For example, the bone mass of a long bone

may be considered as the amount of ash per unit length of bone where the volume is the total volume enclosed by the periosteum. Alternatively, it could be considered as the amount of bone per unit mass of cortex. Because the internal and external diameters vary with age, these measurements give very different results when considering the relative rates of change of bone mass with age.

A further consequence of the failure to define osteoporosis quantitatively has been the failure to measure and report some parameters of bone mass in the majority of publications dealing with various aspects of this condition. The patients are designated as having osteoporosis, but no associated measurements are given to indicate the amount of bone present, or how this relates to the normal population.

The first Chapter of this thesis is therefore concerned with the definition of what is measured by the X-ray methods employed in this study. These measurements are used to examine the changes which occur in the normal male and female population. The following factors which are thought to affect the amount of bone present have been considered: first, the effects of age; second, the differences between the sexes; third, the influence of social class; fourth, the effect of the menopause in women.

The study of the normal population cannot of itself lead to a definition of osteoporosis and this is considered further in relation to the bone changes occurring in pathological states in Chapter II. However, the definition of osteoporosis would not be complete without a discussion of the relation between the bone mass and the incidence of fractures. This is considered in Chapter III. It has been known for some time that the incidence of fractures increase with age (Knowelden, Buhr and Dunbar, 1964), and that there is a fall in bone mass with age (Gitman and Kamholtz, 1965; Epker, Kelin and Frost, 1965; Smith and Rizek, 1966; Nordin, MacGregor and Smith, 1966; Morgan, Spiers, Pulvertaft and Fourman, 1967; Smith, Anderson, Shimmins, Speirs and Barnett, 1969). The relation between bone loss and fracture incidence is discussed by Newton-John and Morgan (1968). They were able to demonstrate a relation between fracture incidence and the amount of bone in the metacarpal in women relating to fractures of the neck of femur and lower end of the radius. However, both the incidence of fracture and the bone loss increases with age, and these authors were careful to point out that correlation did not imply causation. Surprisingly little attention has been paid to

the relative rates of increase in fracture incidence and bone loss in men. The incidence of fracture varies between the sexes and if one particular measurement of bone mass defines the incidence of fracture in both sexes, the case for bone loss being the cause of fracture is considerably strengthened. These relations are examined in Chapter III.

2) Factors affecting calcium balance

It is self-evident that any subject who is losing bone must be in negative calcium balance. It is also evident that the negative calcium balance could be the cause or the result of changing bone mass. Clearly, therefore, the estimation of calcium balance is theoretically a useful technique in the investigation of disorders of calcium metabolism.

The state of the calcium balance in any individual may be the manifestation of low calcium intake, diminished calcium absorption, increased urinary calcium excretion, excessive endogenous faecal calcium loss, or loss associated with sweating. However, the measurement of calcium balance is both tedious and time consuming and only measures balance over relatively short periods of time. The balance technique of itself measures only net faecal loss and excessive loss of endogenous calcium can only be inferred if the faecal losses exceed the dietary intake. It takes no account of sweat losses. The measurements can only be made in relatively small numbers of patients. Finally, the balance technique is subject to error of between 5 and 10% and may therefore

be more useful in relative than in absolute measurement. A further important consideration is that balance may be a function of intake, and may not, therefore, give an indication of the effect of variations in intake which occur over long periods of time.

Chapters IV and V are concerned with the investigation of isotopic measurement of calcium absorption, and the factors controlling urinary calcium excretion.

3) Bone formation and destruction rates

Whether negative calcium balance is the cause or effect of bone loss, it is evident that the rate of bone formation must be lower than the rate of bone destruction if the bone mass is diminishing. Albright, Smith and Richardson (1941b) and Albright and Reifenstein (1948) concluded that osteoporosis resulted from the failure to lay down bone matrix and was therefore the result of diminished bone formation. However, more recently, this view has been challenged. Jowsey (1966) using a quantitative microradiographic technique claimed that the rate of bone formation was normal in osteoporosis but that the rate of bone destruction was increased. Moreover, with the introduction of ^{45}Ca, ^{47}Ca and ^{85}Sr, a number of authors were unable to find any significant difference in the rate of bone formation, measured isotopically, between normal subjects and osteoporotic subjects (Fraser, Harrison and Jones, 1960; Dymling, 1964; Nordin, Smith and Glass, 1964; Lafferty, Spencer and Pearson, 1964).

However, there are a number of problems associated with the use of isotopes to measure the bone formation rate. Quantitatively, the results differ substantially from those obtained using the tetracycline labelling technique.

Bauer, Carlsson and Lindquist (1961) give a value of about 18% of the skeleton being formed per year in adults using isotopes. Frost (1963) measured the rate of bone formation in man after tetracycline administration. This author's measurements were made mainly on rib and the values he obtained ranged between 2 and 7% per year. These differences arise from several factors which are inherent in the use of isotopes for the measurement of bone formation. Following the injection of an isotope the tracer is distributed throughout a rapidly exchanging pool of about 5 gm which is partly associated with soft tissue calcium, and partly with bone surfaces. With time the isotope is steadily lost from this pool, and has little significance after 40 to 50 days (Smith, Speirs and Shimmins, 1967). Long-term exchange processes are also responsible for isotope uptake (Shimmins and Smith, 1966; Smith et al, 1967). By exchange is meant the exchange of isotope for stable calcium without any net acquisition of calcium by the bone. These processes affect the calculation of bone formation which is basically derived by dividing the skeletal retention by the integrated plasma specific activity.

Both retention and plasma specific activity are affected by exchange.

The magnitude of these effects have been examined by a number of authors (Marshall, White and Cohen, 1959a; Marshall, Rowland and Jowsey, 1959b; Marshall, Jowsey and Rowland, 1959c; Marshall, Rowland and Jowsey, 1959d; Lloyd, 1965) using the techniques of quantitative auto-and micro-radiography in animals, and by Rowland (1963) who studied the retention of radium ingested by human subjects. Estimates of the retention of isotope due to exchange processes range from 10 to nearly 50%, depending upon the time since injection of the isotope and the animal investigated. This means that the exchange processes could affect the measured rate of bone formation to a considerable and varying extent.

Further problems arise with the use of ^{85}Sr as a tracer for calcium. Discrimination is known to occur in the transport of these ions across the gut (Bailey, Bryant and Loutit, 1960; Spencer, Li, Samachson and Laszlo, 1960; Samachson, 1963; Nordin, Smith, MacGregor and Nisbet, 1964b).

There is also discrimination against strontium during reabsorption by the renal tubule (MacDonald, Noyes and Lorick, 1957; Harrison, Lumsden, Raymond and Sutton, 1959; Barnes, Bishop, Harrison and Sutton, 1961). Whether there is discrimination at the bone surface during exchange process or new bone formation, or the process of bone mineralisation, is still controversial.

These problems have been examined in Chapter VI, which is in two parts. Part I is concerned with the isotopic measurement of mineral into bone; Part II is concerned with the isotopic measurement of bone resorption and the relation between this and urinary hydroxyproline excretion. The effects of age on these measurements in women have also been investigated.

4) The role of dietary intake of calcium, phosphorus, protein and vitamin D in relation to bone mass and age; the absorption of calcium and urinary calcium excretion; the role of calcium supplementation of the diet

The role of calcium in the development and treatment of osteoporosis has been a topic of debate for nearly a century. Calcium deficient diets were given to animals by a number of researchers (Weiske, 1874; Miwa and Stoeltzner, 1898; Gotting, 1909). These diets were given in an attempt to produce rickets, but Pommer (1925) reviewed this work and concluded that calcium deficiency produced osteoporosis and not rickets. Jaffe, Bodansky and Chandler (1932) reported osteoporosis in dogs and Bell, Cuthbertson and Orr, (1941) reported it in rats fed low calcium diets; this work was confirmed by Crawford, Gribetz, Diner, Hurst and Castleman (1957). Bussaberger, Freeman, Smith and Ivy (1938) reported severe osteoporosis produced by gastrectomy in puppies, which was presumed to be due to malabsorption of calcium. Melanby (1918) reported the development of osteoporosis induced by giving large doses of phytate, which decreases calcium absorption, but with adequate vitamin D. The osteoporosis so produced

could be prevented by administering adequate calcium. Jowsey and Gershon-Cohen (1964) produced osteoporosis in cats with low calcium diets, and they were able to improve the condition by increasing the dietary calcium intake.

The evidence for calcium being of importance in the treatment of osteoporosis in man remains controversial. A positive calcium balance resulting from calcium supplementation of the diet has been widely reported (Owen, Irving and Lyall, 1940; Anderson, 1950; Whedon, 1959; Harrison and Fraser, 1961; Nordin, 1962a). However, Smith and Frame (1965) were unable to demonstrate any relation between vertebral density and calcium intake in 2,000 women. Dent and Lyal Watson (1966) are sceptical of the significance of calcium supplements being an effective therapy. Certainly no evidence of an increase in bone density after administration of calcium therapy has ever been reported.

Surprisingly little attention has been paid to the possible importance of phosphorus in relation to the development of osteoporosis. Investigations that show that changes in phosphorus intake produce significant clinical effects have aroused little controversy. Bergstrand (1921) demonstrated

the development of parathyroid hyperplasia in association with the phosphate retention of renal failure. Stoerk and Carnes (1945) demonstrated a rise in the weight of the parathyroid glands of animals given a high intake of phosphorus. Goldman and Bassett (1958) showed that a high phosphate intake was associated with a rise in the plasma phosphorus and a rise in the filtered load, but a fall in reabsorption by the renal tubule which they attributed to parathyroid stimulation. Similar results were obtained by Crawford, Osborne, Talbot, Terry and Morrill (1950).

The effects of hyperparathyroidism on bone are well-known. Hossain, Smith and Nordin (1970) showed that the cortical area/total area ratio was significantly lower in the post-menopausal patients with hyperparathyroidism than in either pre or post-menopausal women with hypoparathyroidism or in pre-menopausal women with hyperparathyroidism. Jowsey (1966) showed both increased bone formation and destruction in primary hyperparathyroidism, but the increase in destruction rate exceeded the rate of bone formation. The amount of phosphorus in the diet, could therefore be a contributory factor to changing bone mass by its influence on the parathyroids.

Wachman and Bernstein (1968) suggested that since phosphate was the main buffer system, the intake of high acid ash diets might contribute to phosphate loss and therefore to loss of bone.

A number of authors have reported diminished bone growth in animals fed on a low protein diet (Gardner, 1945; Armstrong and Estremera, 1948; McCance, 1960; Dickerson and McCance, 1961; El-Maraghi, Platt and Stewart, 1965; Platt and Stewart, 1962; Shenolikar and Narasinga Rao, 1968). It is possible to have stunting of growth but to have relatively normal bone per unit volume. It is not always clear from the work reported above whether there has been merely failure to grow or whether there has also been a diminution in the amount of bone per unit volume. Gardner (1945) and Armstrong and Estremera (1948) reported substantial falls in body weight but only small falls in the weight of the skeleton. However, El-Maraghi et al (1965) found that low protein diets did cause osteoporosis, irrespective of the calcium content of the diet. A low protein diet increased faecal calcium excretion (McCance, Widdowson and Lehmann, 1942; Shenolikar and Narasinga Rao, 1968). The latter authors concluded from their studies that this was due to increased excretion of endogenous faecal calcium.

Garn, Rohman, Behar, Viteri and Guzman (1964) reported that children who had been admitted to hospital with acute protein-calorie deficiency showed marked deficiency of cortical bone. The degree of ossification was similar to that of the population from which these children were drawn. They felt that this evidence was in keeping with bone loss and was not simply the result of failure to grow. The measurement they used was the cortical width, and this was not therefore corrected for size. On the evidence given, these differences could have arisen from a primary failure of growth. Moreover, there was no evidence given which would exclude vitamin and mineral deficiencies as contributory factors. In fact, the evidence for pure protein deficiency producing osteoporosis in man is very poor. All populations in whom the intake of protein is low invariably have deficiencies of other important dietary constituents. Albright, Forbes, Bartter, Reifenstein, Bryant, Cox and Dempsey (1950), and Albright, Bartter, Dempsey, Forbes, Henneman and Reifenstein (1953) claimed that albumin infusions improved calcium balance in osteoporosis and Dent and Lyal Watson (1966) reported a positive balance in one patient so treated. The significance of low protein diets in the production of osteoporosis in man therefore remains doubtful.

Rickets in children was a well-known clinical entity when this was described by Whistler in 1645.

Trousseau (1862) used fish oil as a remedy for rickets in children and reported the findings of Bretomean in 1827 when a dramatic cure was effected in one of his young patients.

In 1868, Gee (quoted by Franklin, 1954) was convinced of the therapeutic value of cod-liver oil. Palm (1890) was the first researcher to suggest that lack of sunlight might be a contributory factor in the development of rickets.

Mellanby (1918,1921) was able to produce rickets in a young dog and then effect a cure with cod-liver oil. This was also investigated in rats by McCollum, Simmonds, Parsons, Shipley and Park (1921) and McCollum, Simmonds, Shipley and Park (1921). Kandutsch, Murphy and Dreisbach (1956) were able to show that 7-dehydrocholesterol found in the sebaceous glands of the mouse and the guinea-pig produced vitamin D_3. The significance of vitamin D in the development and treatment of rickets and osteomalacia is evident. Further investigations have shown that vitamin D increased calcium absorption (Underwood, Fisch and Hodge, 1951; Nicolaysen and Eeg-Larsen, 1956; Haavaldsen and Nicolaysen, 1956; Gershoff and Hegsted, 1956; Schachter and Rosen, 1959). The general conclusion was that

vitamin D increased calcium absorption in the upper small intestine. Vitamin D is also reported to increase the absorption of phosphorus from the gut (Carlsson, 1954; Nordin and Smith, 1967). This effect may be secondary to increased calcium absorption as Nicolaysen (1956) was unable to demonstrate increased phosphorus absorption in the absence of dietary calcium. However, Nordin and Smith (1967) showed an increased absorption of phosphorus in patients with treated osteomalacia before the calcium absorption responded to vitamin D.

Vitamin D is also known to have a direct effect on the reabsorption of phosphorus by the renal tubule. In large doses, in the treatment of hypoparathyroidism, vitamin D decreases the reabsorption of phosphorus (Albright and Reifenstein, 1948). In smaller doses, in the treatment of rickets and osteomalacia, vitamin D decreases phosphate excretion due to the suppression of secondary hyperparathyroidism (Harrison and Harrison, 1941; Crawford, Gribetz and Hurst, 1954; Nordin and Smith, 1967).

Vitamin D is known to have a direct effect on the metabolism of bone quite apart from the indirect effects it might have by affecting the absorption and excretion of calcium and phosphorus.

Progressively increasing doses of vitamin D will eventually result in a rise in plasma calcium without significantly increasing calcium absorption (Lindquist, 1952; Carlsson and Lindquist, 1955). This almost certainly explains the excessive resorption of bone when vitamin D is administered in large doses (Follis, 1955; Crawford, Gribetz, Diner, Hurst and Castleman, 1957), the action being a direct one on the bone.

The role of vitamin D in the development of osteoporosis remains obscure. Although not attributing a specific role to vitamin D deficiency in the treatment of osteoporosis, most researchers would recommend an adequate vitamin D intake, usually with the comment that this should be given to ensure adequate absorption of calcium (Fourman, 1963). However, Rose (1964) demonstrated that though vitamin D increased calcium absorption, there was a significant rise in urinary calcium which resulted in only a small or negligible improvement in balance. Smith, Rizek, Frame and Mansour (1964) estimated the serum anti-rachitic activity (SARA) in 116 women from Michigan and 65 women from Puerto Rico. All the women were over 45 years of age.

The serum antirachitic activity was assayed on rachitic rats. The state of the spinal density was assessed by two independent observers on a 4 point scale - normal, grade 1, grade 2 and grade 3. Grades 2 and 3 were taken to indicate severe osteoporosis. In the subjects from Puerto Rico, the SARA was significantly higher than in the subjects from Michigan. There was a seasonal variation, the SARA level in the Michigan subjects being higher at the end of the summer than in the spring. The SARA level was lower in the older Michigan subjects and in the women with severe degrees of osteoporosis compared with those subjects who had normal spinal densities or grade 1 osteoporosis. . The serum calcium and phosphorus values were also found to be higher in the subjects from Puerto Rico. Smith et al (1964) suggested that the lower SARA values in the Michigan subjects could result from the lower exposure to sunlight, and that this might affect the older women in particular in whom the sebaceous secretion of the skin was diminished.

Seasonal variations in serum phosphorus have been reported in children (Hess and Lundagen, 1922), and in serum calcium values in infants (Bakwin and Bakwin, 1927). Similar variations in serum phosphorus in healthy men have been reported by Havard and Reay (1925). In subjects with a good dietary intake of calcium, vitamin D was not found to affect calcium balance (Bogdonoff, Shock and Nichols, 1953). In fact, in other studies vitamin D has been reported to improve calcium balance in elderly men and women (Shorr and Carter, 1947; Ackermann and Toro, 1953; Lutwak and Whedon, 1963). The evidence is therefore far from conclusive that vitamin D is a contributory factor in the development of osteoporosis in some subjects, but is sufficient to suggest that further investigation is important.

These problems have been considered in Chapters VII, VIII, IX and X.

In Chapter VII, the relation between dietary intake and bone mass has been examined in normal men and women. In addition an isotopic test of calcium was used to assess the differences in absorption which might be associated with age and sex. The urinary calcium/creatinine ratio was used to assess the changes in urinary calcium excretion.

Chapter VIII is concerned with the assessment of dietary intake of calcium and phosphorus in patients who had undergone gastric surgery, and the relation of diet to bone mass.

The method of measuring bone mass on the third metacarpal proved sufficiently accurate and reproducible to permit a study of the effects of calcium supplements on bone mass. These results are reported in Chapter IX.

The final chapter (Chapter X) is concerned with a further examination of the effects of calcium supplementation on balance, faecal excretion of calcium, fat and bile acids, and the urinary excretion of hydroxyproline. This was then considered in relation to the study of the effects of calcium supplements in man.

The conclusions based on the experimental evidence are stated under this heading (p. 270).

Finally, there are four Appendices which though not a direct part of the investigations, are concerned with describing more fully the methods used where these are not well established routine techniques.

CHAPTER I

BONE MINERAL CONTENT IN HEALTH AND IN DISEASE

1. Bone mineral content in normal male and female subjects

Attempts to study the effects of age, diet and the many other factors affecting calcium balance, and therefore the mineral content of bone in health and in disease have had to await the development of accurate methods of estimating small percentage changes. Numerous attempts have been made to define a simple and reproducible technique. These fall into two broad groups. Firstly, there are the morphological measurements such as the cortical width of the metacarpal, femur and radius (Barnett and Nordin, 1960; Meema, 1963) and the measurement of spinal biconcavity (Barnett and Nordin, 1960). Secondly, many attempts have been made to estimate the density of various bones: the spine (Nordin, Barnett, MacGregor and Nisbet, 1962); the ulna (Keane, Speigler and Davis, 1959; Doyle, 1961); the radius (Meema, Harris and Porrett, 1964); the os calcis (Mayo, 1961), and the metacarpal (Bywaters, 1948; Koch and Kaplin, 1961). The morphological methods have the advantage of simplicity but only a moderate degree of accuracy. Moreover, morphological measurements do not allow for changes in bone porosity which occur with age and in osteoporosis (Jowsey, 1963a; Jowsey and Gershon-Cohen, 1964) nor for the changes in mineral content without loss of bone tissue, for example, in

osteoporosis (Jowsey and Gershon-Cohen, 1964; Dollerup, 1964), or even more obviously in osteomalacia. Morphological methods are insufficiently accurate to make an assessment of therapy where a 5% change implies a substantial loss of bone and which is less than the error of measuring the cortical width of a bone such as the metacarpal (Anderson, Shimmins and Smith, 1966).

Methods

A combination of morphological and density measurements give much more information about the skeleton than either type of measurement taken alone. In the following study, such a combination has been used, and the methods and the degree of accuracy and reproducibility are more fully described in Appendices I and II. The morphological methods are those described by Barnett and Nordin, 1960. Standard films were obtained of the right hand and the left femur (antro-posterior) and a lateral tomogram of the spine. The standard films were used to calculate the osteoporotic indices, the measurements being made with a micrometer caliper. The ratios of the total cortical width to shaft diameter was determined at the midpoint of the second metacarpal of the right hand for the Metacarpal Index (M.I.), and at the widest part of the cortex of the femoral shaft for the Femoral Index (F.I.). The lumbar vertebral body biconcavity index (S.I.) was measured on the tomogram

of the third lumbar vertebrae. This is the ratio of the vertical height at the centre of the body divided by the vertical height of the anterior border. The Relative Vertebral Density (R.V.D.) was measured from a tomogram of the third lumbar vertebrae, the patient and standard spine being X-rayed side by side on the same film. This method was developed by Nordin et al (1962) and is described in Appendix II.

In order to obtain a more accurate and reproducible measure of the mineral content of bone, which was especially important if the effects of treatment were to be followed, a method for measuring the density and mineral content of the 3rd metacarpal was developed and is reported elsewhere (Anderson et al, 1966). A description of the method is given below and the technique detailed in Appendix I. Initially, the aluminium equivalent of the midpoint of the 3rd metacarpal is determined by measuring the absorption of X-rays at this point. To do this, the hand is placed alongside an aluminium step-wedge, and an X-ray film taken under standard conditions. The conditions are imposed in order to eliminate variations due to the X-ray source (the 'heel' effect), and those due to scattered radiation.

Non-screen film is used to obtain improved definition, and to eliminate variations consequent upon the non-uniformity of response of the screen (Doyle, 1961). The screens were removed from screened film for this purpose as no non-screen film suitable for automatic processing was available. The aluminium step-wedge and hand were X-rayed on the same film to reduce variations due to exposure and development of the film. The aluminium equivalent is determined making allowance for soft tissue replaced by bone and for marrow which has an X-ray absorption significantly different from soft tissue. The 3rd metacarpal was chosen in preference to other bones because it is relatively easy to measure on the X-ray and is reasonably uniform in shape. Further, the soft tissue mass is uniform and relatively small in amount so that baseline variation and scatter are minimal.

From the aluminium equivalent, four further measurements of bone mineral content were made:

1) The standardised aluminium equivalent (S.A.E.). This is derived by dividing the aluminium equivalent of the bone in the metacarpal by the width of the bone at the point at which the measurement was made. This allows for the difference in sizes between individuals, and is a measure of the mineral per unit width in the whole metacarpal. It is, therefore, a measure of whole bone density (Fig. 1).

2) Lambda (λ). This is the absorption coefficient of the metacarpal cortex and is found by dividing the aluminium equivalent by the cortical width at the midpoint of the metacarpal. This is therefore a measure of the density of the cortical bone (Fig 1).

3) Total cortical mineral (T.C.M.). This is the total mineral per unit length of cortical bone. This is derived by multiplying the cortical density (λ) by the cortical area $\pi (r_1^2 - r_2^2)$ where r_1 is the radius measured to the periosteal surface and r_2 the radius measured to the endosteal surface (Fig 1).

4) Standardised total cortical mineral (S.T.C.M.). This is derived by dividing the total mineral content (T.C.M.) by the square of the total width. This again makes allowance for the difference in sizes between individuals (Fig 1).

Subjects studied

Volunteers were obtained by approaching the relatives and friends visiting patients in the general medical wards of the Western Infirmary, Glasgow. The scope of the survey was explained to them, and with their full permission X-rays were taken and a standard questionnaire completed. The questionnaire was designed (Appendix III) to elicit the incidence of fractures, backache, any history suggestive of malabsorption or diarrhoea, gastric surgery, corticosteroid therapy, the occurrence of renal colic or stone, and symptoms suggestive of urinary tract infection.

A past or present history of endocrine disorders was queried. Subjects with secondary bone disease (Nordin, 1961) were excluded from the normal survey.

In addition, the age of the subjects was noted. The social class was recorded, the social class of a woman being that of her husband if married or her own if single or widowed. The social class was determined from Appendix B1 of the Registrar General's Classification of Occupations (1960).

In the female subjects, the time of onset of the menopause, defined as the age at the time of the last period, was noted and whether it had occurred naturally or as the result of hysterectomy or oophorectomy. The number of pregnancies and the incidence of breast feeding was recorded.

Four separate normal groups were studied. Group I consisted of 152 normal women studied between 1961 and 1963. Group II consisted of 75 male subjects studied between 1963 and 1966. In these groups the X-ray measurements made were of the Metacarpal Index, Femoral Index, Spinal Index and the Relative Vertebral Density. Dietary histories were

were taken and an isotope test of calcium absorption was carried out. Two urine samples were taken and the calcium/creatinine ratio estimated for each. The mean of the two readings was calculated and recorded.

Two further groups of subjects were studied between 1964 and 1966. These consisted of 317 female and 312 male volunteers. These subjects were recruited in the way described above, and a standard questionnaire completed as described in Appendix III. The social class of the subjects was noted, and with their permission an X-ray of the right hand was taken along with an aluminium standard as described in Appendix I. From the aluminium equivalent of the bone, the S.A.E., λ, T.C.M. and S.T.C.M. were calculated.

RESULTS

Social class

The recorded social classes of the subjects in Groups I, II, III and IV are shown in Table I. The frequency distribution of the numbers in each of the five social classes is shown for each group and the percentage of subjects in each class indicated. The final columns show the breakdown into social classes of the Central Clydeside Conurbation. In Group I (normal female) there is a preponderance of social class 3 which is reflected in the patients referred for investigation of osteoporosis (p. 193) and probably reflects accurately the population served by the hospital at that time. In Groups II, III and IV the distributions still show a preponderance of subjects in social class 3 though this more nearly approaches the distribution within the Central Clydeside Conurbation (Appendix B1 of the Registrar General's Classification of Occupations, 1960). This difference is thought to reflect some of the change in occupation of the subjects studied, and also the re-housing of subjects associated with the demolition of many of the older houses in the area.

Age distribution and incidence of backache

The frequency distribution by age of the subjects in 5 year age groups and the incidence of backache in each group is shown in Figs. 2, 3, 4 and 5. There is a higher incidence of backache in the female subjects in Group I than Group III at all ages but the differences do not reach statistical significance. The male subjects in Groups II and IV and have a lower incidence of backache than the female subjects in Groups I and III, again at all ages.

Menstrual history in Groups I and III

In Group I the menstrual history was not recorded in nine of the subjects. Eight of the subjects had had either oophorectomy or a hysterectomy sometime between 27 and 56 years of age, the average age being 40 years. The mean age of the 81 women in whom the menopause had occurred naturally was 47 years.

In Group III, the menstrual history was not recorded in ten of the subjects. Nineteen of the subjects had undergone oophorectomy or hysterectomy between 23 and 66 years of age, the average age at operation being 45.7 years. The mean age of the menopause in the 160 women who had undergone a natural menopause was 47.8 years.

Other medical history

The incidence of other medical conditions present in the subjects who volunteered are shown in Table II. The incidence of urinary symptoms are higher in the female Groups I and III. A higher proportion of subjects in Groups II and IV had had gastric surgery than would have been expected, but the proportions were small - 0.6% and 1.2% - and would not be expected to significantly affect the normal distribution.

X-ray measurements

(a) Group I (152 normal female subjects) and
Group II (75 normal male subjects)

Group I (Normal females)

There was a highly significant fall in the Metacarpal Index (M.I.), the Femoral Index (F.I.), the Spinal Index (S.I.), and the Relative Vertebral Density (R.V.D.) with age (Table III). The gradient was steepest in the fall in the R.V.D. followed closely by the fall in the M.I.. The smallest and least significant change was found in the Spinal Index ($P < 0.005$). The rate of fall in the Femoral Index ($P < 0.001$) was intermediate between that of the M.I. ($P < 0.001$) and S.I. ($P < 0.001$).

Group II (Normal males)

There was a significant fall in the Femoral Index (Table III) with age (Fig. 115), ($P < 0.01$). Though both the Metacarpal Index and the R.V.D. showed a tendency to fall with age, this did not reach statistical significance (Figs. 114 and 117). There was no fall in Spinal Index with age (Fig. 116). The M.I. and the R.V.D. were significantly lower in the female subjects of group I above the age of 60 years when compared with the male subjects of group II in the same age range (Figs. 114 and 117). No significant difference between the sexes was found in either the F.I. or the S.I. (Figs. 115 and 116).

In group I, (Figs. 6, 7 and 8), the changes in the R.V.D. M.I. and F.I. are related to the menopause. The X-ray index and density is plotted in relation to age in those women who have not yet reached the menopause. In those individuals who have passed the menopause, the bone mineral measurement is plotted against the years since the menopause occurred. The data has been analysed in 5 year groups and the mean and two Standard Error (S.E.) range is shown. There is no significant change in either the R.V.D. or the M.I. before the onset of the menopause. By the second 5 years of the onset of the menopause, the R.V.D. falls significantly (Fig. 6). In Fig. 7, it can be seen that a similar change has been found in the M.I. but the change is apparent 5 years later. A similar fall was found

in the F.I. 15 years after the onset of the menopause (Fig. 8). There was no point at which an abrupt change in the Spinal Index occurred.

(b) Group III (317 normal female subjects) and
Group IV (312 normal male subjects)

The volunteers for these two groups were recruited in the same way as those taking part in Groups I&II. This survey was carried out between 1964 and 1966. The questionnaire used was the same as that for the survey of Groups I and II and is described in Appendix III.

The X-ray measurement was a single X-ray of the right hand along with an aluminium standard from which the Aluminium Equivalent (A.E.), Standardised Aluminium Equivalent (S.A.E.), Cortical density (λ), Total Cortical Mineral (T.C.M.) and Standardised Total Cortical Mineral (S.T.C.M.) were derived (see p. 30).

The data was first analysed in terms of the mean and two S.E. range in 5 year age groups. In Group III (normal females), the S.A.E. rose from 20.9 between the ages of 5 and 9 years to a mean value of 36.5 between 25 and 29 years of age (Fig. 9). The mean S.A.E. fell slowly thereafter to a value of 34.7 between

45 and 49 years, and then more rapidly to a value of 24.1 between 70 and 74 years. The S.A.E. has been related to the menopause (Fig. 10). There is an abrupt fall in the S.A.E. 5 years after the onset of the menopause (which occurred at the mean age of 47.8 years in this group) and much more slowly thereafter. In the male subjects (Group IV), the S.A.E. rose from a mean value of 21.1 between 5 and 9 years to 35.5 between 30 and 34 years (Fig. 11). There was then a gradual fall to 29.7 between 70 and 74 years. At no five year interval was there any abrupt change in S.A.E. comparable to that seen in the female subjects (Group III), which is evident between 50 and 54 years (Fig. 9). The differences between the male and female subjects in corresponding age groups have been tested for significance using Student's 't' test. The results are shown in Table IV. No significant difference arises until after the age of 64 years and thereafter the gap between the male and female subjects continues to widen.

On examination of the data, it is evident that there is an initial sharp rise and then a fall and finally the data tends to flatten with increasing age. Because of this, a third order polynomial has been fitted to the data to show the continuous

change with age. Since a third order polynomial might place an unnatural constraint upon the data this was tested by applying the polynomial starting at various decades between 5 and 45 years. No significant change in distribution was apparent. In Fig. 12, the mean and 95% Confidence limits for the data are shown for female subjects (Group IV) by an interrupted line, and for the male subjects (Group III) by a continuous line. The mean values taken from the line of best fit show a rapid rise in the S.A.E. from just above 20 at the age of 5 to the age of 20 for both the male and female subjects. The value for the female subjects starts just below the value for the males, rises above the value of the male subjects by the age of 10 and remains above until the age of 48 years. Thereafter there is a more rapid fall in the values for female subjects taking them below the value for male subjects reaching a value of 23 at 75 years. The highest mean value for the female subjects is 36.34 which is reached at 34 years of age. The male subjects reach a peak mean value of 35.4 at 36 years of age. Thereafter there is a much slower fall in the male subjects to a value of 29 at 75 years.

The value λ, which is a measure of the density of the cortical bone, has been measured in both Groups III and IV. Initially, the data was analysed in 5 year age groups and the mean and two S.E. range is shown in Figs. 13, 14 and 15. In the female subjects, the density rises from a mean value of 4.4 between 5 and 9 years to reach a peak of 5.8 between 30 and 34 years, and falls slowly thereafter (Fig. 13). The relation to the menopause has been tested but no significant fall in density was found to be associated with it (Fig. 14). In the male subjects, the mean density is 4.8 between 5 and 9 years (Fig. 15) rising rapidly to a value of 5.6 between 25 and 30 years and then rising slowly to a value of 5.8 between 70 and 74 years. The differences between male and female subjects has been tested for significance using the Student's 't' test, but no difference was found between the sexes except between 70 and 74 years when the difference reached significance at the 5 percent level, the female subjects having a lower density at this age (Table IV).

A third order polynomial was again fitted to the data and the results are illustrated in Fig 16. The mean and 95% Confidence limits are shown for the data, the results for the male and female subjects being superimposed so that they can be compared. The female subjects have a λ of 4.49 at 5 years rising rapidly to 5.8 at 25 years and then more slowly to a maximum value of 5.84 between 37 and 42 years, and falling slowly to a value of 5.65 at 75 years. The male subjects (Group IV) showed a rapid rise from 4.76 at 5 years to a value of 5.40 at 25 years and then a continuing slow but steady rise to 5.93 at 75 years. Both the male and female subjects showed a further small rise in density after the age of 75 years, but the significance of this is questionable, as the numbers were small. The value of λ for the female subjects rises above that for the male subjects at 13 years (λ = 5.13) and falls below the value for the male subjects at the age of 61 years (λ = 5.68).

The total cortical mineral (T.C.M.) and the standardised total cortical mineral (S.T.C.M.) also vary with age. The results between 30 and 75 years of age are illustrated in Figs 17 and 18. The values are those obtained in the normal females (Group III) and in the male subjects (Group IV).

The T.C.M. rises from an initial value of 0.5 in female and 0.75 in male subjects at the age of 5 years to 2.4 at 40 years in the female subjects and to a value of 2.9 again at 40 years in the male subjects. Since the T.C.M. is a measure of the total cortical mineral per unit length, and as the male subjects are on average larger than the female subjects, they would be expected to have a larger amount of mineral than the females. After reaching a peak, the T.C.M. shows a small decrease in the male subjects up to the age of 75 years of 4%. The female subjects show a steady fall in T.C.M. after the age of 40, the fall being 25% between 40 and 70 years. The data is analysed in terms of a third order polynomial and the results for both the male and female subjects between 30 and 75 years are shown in Fig 17. The mean values at one yearly increments are also shown.

The standardised total cortical mineral has been analysed in exactly the same way and the results are illustrated in Fig 18. These show the change in the standardised total cortical mineral with age in the normal female and male subjects.

The third order polynomial fit for both the male and female subjects is shown in Fig 18. Both methods of analyses, i.e. taking the mean value at yearly intervals, and analysis by third order polynomial, show good agreement. The S.T.C.M. reaches a peak value of 3.8 between 30 and 35 years in the female subjects, and 3.6 between 35 and 40 years in the males. The males then fall to a value of 3.3 between 70 and 75 years, the fall tending to flatten off at 70 years. The females show a much greater fall to a value of 2.8 at 75 years and have a higher S.T.C.M. than the males until the age of 50 years. The difference reaches statistical significance at the 5 percent level between the age of 60 and 65 years, the significance of the difference increasing thereafter.

Changes in external diameter, medullary width and cortical area with age

This has been considered in the two largest groups, i.e. Groups III and IV. These consist of the 317 normal female and 312 normal male subjects. There is a steep rise in the external diameter from 5.9 at 5 years of age to 7.80 mm at 30 years in the female subjects. In the male subjects this rise is from 6.1 at 5 years to 8.95 mm at 30 years. The changes between 30 and 80 years are illustrated in Fig 19.

In the female subjects there is a small continuing rise between 30 and 50 years from 7.80 to 8.15 mm and a small fall back to 8.05 mm at the age of 75 years. These changes are not significant. In the male subjects, there is a slow but steady rise from 8.95 mm at 30 years to 9.2 mm at 75 years, an increase of 1.6% which again does not achieve significance.

In both the female and the male subjects, the medullary width increases sharply between 5 and 30 years from 2.5 mm to 3.3 mm in the females and 2.6 mm to 3.4 mm in the males. In the female subjects, the width increases from 3.3 mm to 4.9 mm (53.9%) between 30 and 75 years. In the male subjects this rise is from 3.4 mm to 4.8 mm (32.7%). These changes are highly significant ($P < 0.001$). The changes in medullary width between 30 and 80 years for both male and female subjects are illustrated in Fig 20. These changes in external and internal diameter have a significant effect on the cortical area.

It is the combination of these changes in cortical area and density (λ) which account for the characteristic distribution of T.C.M. and S.T.C.M. shown in Figs 17 and 18. These changes also affect the Aluminium Equivalent (A.E.) and the Standardised Aluminium Equivalent (S.A.E.) to cause the changes between 30 and 80 years illustrated in Figs 21 and 22.

Mineral content and social class

There is a preponderance of social class 3 in all four groups. This is most evident in Group I in which the percentage of patients in social class 3 is greater than that of the population as a whole. The number of patients in social class 3 in Groups II, III and IV are slightly below these in the general population.

Since differences in social class may result in differences in nutrition which might in turn affect the bone mineral content, this has been tested. In Table V, the S.A.E. (whole bone density) in the male and female subjects of social classes 1 and 2 have been compared in each sex in 10 year age groups with social classes 4 and 5.

No differences have been found, except

in one group of male subjects between 60 and 69 years of age in whom the subjects from social classes 4 and 5 were seen to have a significantly lower S.A.E. than those individuals in social classes 1 and 2 ($P < 0.01$).

Discussion

The definition of disease is only possible if the limits of normality have first been defined. However, there are many definitions of normal, and where changes occur over many years in the general population and in the patients with disease, it is inevitable that the 'normal' will merge gradually into the abnormal. In this study, the normal subjects are defined as male and female subjects who were approached while visiting patients in the wards of the Western Infirmary, Glasgow. The scope of each survey was fully explained to them and they agreed to take part. In this way it was hoped to secure a cross-section of the population in Glasgow. This does, however, set limits on the population selected.

Firstly, it would be expected that the population selected would reflect only the population served by the hospital and, therefore, need not reflect the population as a whole. In the first survey, which was more detailed than the others, including as it did a medical history, dietary history, absorption test, urinary calcium measurements, and X-ray measurements of hand, femur and spine, the numbers had to be more restricted than for the other surveys which involved only the completion of a questionnaire and an X-ray of the hand. In fact some evidence of selection is evident from the fact that there was a preponderance of subjects belonging to social class 3 in Group I (skilled occupations). In the second survey, Groups II, III and IV, carried out two years later, the percentage of subjects in each of the social classes closely represented those defined by the Registrar General's Classification of Occupations for the country. The distribution of occupations within the social classes in Group I is very similar to that found in a group of hospital patients being investigated for osteoporosis, and the change

in the normal Groups II, III and IV may reflect some movement of the population. There are certain other resemblances between the normal and abnormal populations studied and this is discussed later (p. 193). The other sources of differences in the selection of normal and 'abnormal' population might arise from the fact that the older and less fit relatives and friends might not be able to visit the hospital, and by a refusal of the less fit to volunteer for examination. However, comparisons between the normal and 'abnormal' population discussed later (p. 193) again suggest that the random selection of volunteers is fairly representative of the population as a whole, and the comparison between patients and normal subjects is valid.

Comparison of the medical histories shows that a very similar proportion of the subjects in Group I complained of urinary tract symptoms (32%) compared with those subjects in Group III (30.3%). There was a significantly higher incidence of backache in subjects in Group I (32%) compared with those in Group III (16%). In neither group was there any relation between the incidence of backache and any of the X-ray measurements of bone mineral content.

This merely indicates that there are a great many causes of backache in women, and that osteoporosis may be only one of them. The principal site of the backache was the lumbar spine in both groups.

In the male subjects (Groups II, IV) the incidence of backache was 12.8 and 10.1%, and the incidence of urinary symptoms was 23 and 22.5% which was very much lower than in the female subjects. Again no relationship was found between the incidence of backache or urinary symptoms and the X-ray measurements.

In Group I, there is a fall in all the X-ray measurements with age, the fall being greatest in the Relative Vertebral Density ($R.V.D.$) followed by a smaller fall in the Metacarpal Index ($M.I.$). There is a much smaller fall in the Femoral Index ($F.I.$) and the smallest fall of all occurs in the Spinal Index ($S.I.$) which indicates the degree of spinal biconcavity. In Group II, though there is a fall in the $M.I.$, $F.I.$, and $R.V.D.$, only the fall in the $F.I.$ reaches statistical significance.

In Group III (Females) and Group IV (Males), the amount of mineral in the 3rd metacarpal of the right hand has been measured in four ways. First, the Standardised Aluminium Equivalent ($S_{\circ}A_{\circ}E_{\circ}$) measures the density of the whole bone. Second, is λ, which is a measure of the cortical density (see Appendix I). Third, the $T_{\circ}C_{\circ}M_{\circ}$ which is a measure of the total calcium per unit length of metacarpal. Fourth, the $S_{\circ}T_{\circ}C_{\circ}M_{\circ}$ which is a measure of the $T_{\circ}C_{\circ}M_{\circ}$ divided by the width of the bone to allow for differences in size between individuals.

There is a rapid rise in the whole bone density with age in both male and female subjects over the first 20 years and then reaching a peak at 34 years in the female and 36 years in the male. Thereafter there is a slow, steady fall in the whole bone density of the metacarpal in the male subjects up to the age of 75 years. The female subjects show a decline very similar to that of the males until the age of 50 years when a rapid decline in the whole bone density occurs. The mean $S_{\circ}A_{\circ}E_{\circ}$ for the female subjects remains slightly above that for the males up to the age of 49 years.

A fall in the cortical width with age has, of course, been reported by other workers. Meema and Meema (1963) reported a fall in the width of the humeral cortex in both male and female subjects, the fall starting earlier and occurring more rapidly in the female subjects. Similar results were obtained by Fujita, Orimo and Yoshikawa (1966) in measurements of the metacarpal. Morgan, Spiers, Pulvertaft and Fourman (1967) reported changes in the metacarpal cortical width with age in men and women. They preferred this measurement as they were unable to show a relationship between the marrow cavity and the total diameter.

They also employed a densitometry technique but found it did not give any better results than the simple measurement of cortical width. However, the technique used by them has two sources of error which may have led to a loss of precision. Firstly, no allowances were made for the 'heel' effect and secondly, scatter from the water bath and the bone due to the positioning of the step-wedge would not be uniform over the aluminium step-wedge. This would lead to an unpredictable variation in the X-ray density of the wedge.

We have found greater reproducibility in our densitometric technique than in the simple measurement of cortical width (Anderson et al, 1966 - Appendix I).

Furthermore, as women generally are smaller than men and therefore have smaller bones, a direct comparison can only be made between them if allowance is made for size. This is most usefully done by dividing by the width of the bone or some derivative of this.

Morgan et al (1967) made an estimate of the relation of the calcium content of the metacarpal with age from the X-ray measurements, i.e. a measure of density of the cortex. It is interesting to note that these results resemble those presented here. They found no statistical difference between the male and female subjects over the age range studied, though the cortical density of the metacarpal in the female subjects was slightly higher than in the males. The mean density of the cortex showed a continuing rise with age except within the 7th decade. The female subjects showed a rise until the 5th decade and then fell gradually. Dollerup (1964) measured the mineral content in samples of bone taken from the iliac

crest at post-mortem examination. He found a rapid rise in mineral content up to 20 to 30 years of age. There was a peak at 40 years, but if this is ignored, there was a slow but steady rise with age in subjects up to and over 90 years of age.

The change in density with age is surprising. The female subjects do show a slow fall after the age of 50 years but this is slight. In the male subjects, the rise continues throughout life. A rise in bone mineral density up to the age of 20 years would be expected. Young subjects have a higher proportion of young bone with a higher water content and lower mineral content than mature bone. The density of bone as a tissue will also be affected by the porosity and the degree of mineralisation. Jowsey (1963a) reported an increased intracortical porosity in the femur with age which would tend to cause a fall in density. Epker, Kelin and Frost (1965) found very little change in the porosity of the human rib with age. Change in porosity in the metacarpal with age has not yet been reported. Jowsey (1963b) reported an increasing incidence of plugged and filled lacunae with age.

The plugged lacunae were hypermineralised when compared with surrounding bone, the density being 1.63 gm/cc compared with 1.44 gm/cc in normal bone as shown by Rowland, Jowsey and Marshall (1958) using a quantitative microradiographic technique. This suggests one possible mechanism which may cause an increase in density with age though this is insufficient to do so on its own.

Changes in the total cortical mineral (T.C.M.) with age show a different pattern from the S.A.E.. In the male subject there is no significant change after the age of 30 years. In the female subject, there is a small change between the age of 30 and 45 years, but a significant continuing fall is seen thereafter. The changes with age of the S.T.C.M. in the male and female subjects are similar to those seen in the S.A.E., but the male to female rate of change is different. The differences in the patterns of distribution with age are important because this is of considerable significance in the quantitative definition of osteoporosis and the part it plays in increasing the incidence of fractures in the elderly.

These parameters measure different things and account for the apparent discrepancies in the literature, where bone loss is described as starting at ages ranging from 20 to 50 years (Epker et al, 1965; Takahashi and Frost, 1966; Nordin et al, 1966). It is therefore very important to be absolutely clear as to what is measured by each of the four measurements.

The S.A.E. is a measure of the whole bone density per unit volume, i.e. it is a measure of the density of the metacarpal per unit volume enclosed by the periosteal surface and the marrow cavity is, therefore, included in this volume measurement. λ is a measure of the mineral of the cortex per unit volume. The T.C.M. is the total mineral in the cortex per unit length of the bone. Finally, the S.T.C.M. measures the total cortical mineral per unit length divided by the width of the bone. The S.A.E. and S.T.C.M. allow for the differences in size between individuals so that they may be directly compared. This is necessary as it is obvious

that a lean 13 stone man will have much larger bones and therefore more mineral per unit length than an individual weighing 8 stones, even if neither is osteoporotic. Since all parameters fall with age, it is apparent that the simple qualitative definition of osteoporosis so far applied by many workers in this field is totally inadequate. This problem is examined further in the next two chapters.

CHAPTER II

BONE MINERAL CHANGES IN NORMAL AND PATHOLOGICAL STATES

The normal population has been defined in Chapter I as being the relatives and friends of patients in the general medical wards who volunteered to have X-ray measurements of bone carried out. The reasons for accepting these subjects as a representative sample of the population served by the Western Infirmary, Glasgow, have been discussed.

In the following study bone density measurements in patients referred to a special clinic because they were judged to be osteoporotic, and patients with various metabolic bone diseases, are related to the normal values previously obtained. Though studies of bone density have been made in various conditions, the absence of an adequate quantitative definition of loss of bone mass in relation to the normal population has made assessment of various pathological states difficult. The present Chapter and the following Chapter are therefore concerned with the investigation and definition of normal and pathological bone density.

Bone mineral content in disease

The bone mineral content of the 3rd metacarpal of the right hand has been measured by the technique described in Appendix I (Anderson et al, 1966) in patients with conditions affecting calcium metabolism. The whole bone density ($S_0A_0E_0$) and cortical density (λ) have been determined and are compared with the values obtained in the 317 female (Group III) and 312 male (Group IV) volunteers. In each one of the patients, the questionnaire described in Appendix III was completed.

Subjects studied

The patients investigated were referred to a special Bone clinic or formed part of a special survey. The diagnostic groups were patients referred because they were thought to have osteoporosis, or were known to have fractures of the femoral neck; patients who had undergone gastric surgery for peptic ulcer, and patients suffering from steatorrhoea, osteomalacia and primary hyperparathyroidism. The number of patients in each diagnostic group is shown in Tables VI, VII, VIII and IX.

Diagnostic criteria

The definition of normality has been discussed earlier. That is the normal subjects were volunteers in Groups III and IV.

The 'osteoporotic' subjects were referred to a special Bone clinic because they were thought to have osteoporosis by the referring physician or surgeon on inspection of X-rays of their spines and/or peripheral bones. Each patient had a serum calcium, phosphorus and alkaline phosphatase measured and the urinary calcium was assessed on at least two separate occasions as a calcium:creatinine ratio (Nordin and Smith, 1965). Any history of endocrine disease, gastric surgery or renal stone or diarrhoea led to their exclusion from this group. The patients who had had fractures of the femoral neck or intertrochanteric fractures had also undergone measurements of their serum calcium and phosphorus, and urinary calcium, and had a questionnaire completed. Abnormal findings again led to their exclusion from this group.

The patients who had undergone gastric surgery for peptic ulceration were part of a group who were investigated in greater detail and are discussed later (Chapter VIII). They were patients who had undergone either a polya-gastrectomy or gastroenterostomy with vagotomy 8 to 12 years before.

The patients with steatorrhoea had more than 5 gm of fat per day in their stools. Eighteen of the 29 patients had had jejunal biopsies done and were found to have villous atrophy. Any patients with evidence of osteomalacia as defined by a low serum calcium, low serum phosphorus, raised alkaline phosphatase, low urinary calcium or raised Phosphate Excretion Index were excluded from this group.

Patients with osteomalacia formed a more heterogenous group and consisted of patients who, with and without steatorrhoea, had undergone gastric surgery for peptic ulceration, osteomalacia secondary to idiopathic steatorrhoea and patients who had no evidence of associated pathology, but in whom the dietary intake of vitamin D was low. The definition of osteomalacia rested on the presence of frank osteoid borders on bone biopsy. In addition there was evidence of biochemical osteomalacia (Nordin and Smith, 1965).

The patients with primary hyperparathyroidism all had raised serum calcium, Phosphate Excretion Indices, and the presence of a parathyroid tumour confirmed at operation.

Results

Since marked changes in bone mineral content and density occur with age, it is necessary to allow for this in any assessment of pathological bone loss. In order to do this, a third order polynomial was fitted to the normal data to give a series of lines of best fit to derive the cumulative percentile lines. The 5, 10, 20, 30 and 50 percentile lines are seen in Figs 23 and 24 for the normal female subjects, and in Figs 25 and 26 for the normal male subjects. Tables have also been compiled so that the precise percentile S.A.E. or values for any patient can be determined. A sample of this Table is illustrated in Appendix IV.

Using these values, patients with reported disorders of calcium metabolism are considered in Tables VI to IX. All the Tables are arranged so that the pathological condition identified in the first column, the number of patients in the group in the second column, and the percentile values at the top of the Tables. The cumulative number of patients below each percentile value is shown in the remaining columns.

1. Comparison of the S.A.E. in normal and pathological states

Of the 169 female patients referred to the Bone clinic, 58% were below and 42% above the 50 percentile level (Table VI). They did not differ significantly from the normal population. The male subjects suspected of having osteoporosis showed a very different distribution (Table VII), 83% of the 26 patients being below the 50 percentile level.

Of the 69 female patients with fractures of the neck of femur, though 33.4% were below the 20 percentile, 43.8% were above the 50 percentile (Table VI). Of the male subjects, 50% were below the 20 percentile level and 34.5% above the 50 percentile (Table VII).

Eighteen female and 11 male patients who were found to be suffering from steatorrhoea were assessed in the same way. 83.5% of the females and 73% of the males were found to lie below the 50 percentile level (Tables VI and VII).

Of 86 male patients who had had gastric surgery for peptic ulcer, 74% were found to have an S.A.E. below the 50 percentile level. Only 3 female patients had had gastric surgery, but they were all below the 20 percentile level (Tables VI and VII).

Of 8 female and 3 male patients with osteomalacia, 62.5% of the females were below the 40 percentile level, and all three male subjects were below the 20 percentile level (Tables VI and VII).

Of 6 female and 4 male subjects with primary hyperparathyroidism, a third were above the 50 percentile level (Tables VI and VII).

Of 6 female patients with hypoparathyroidism, 4 were below the 50 percentile level (Table VI).

Comparison of λ in normal and pathological groups

The density measurements in these groups in terms of their distribution in the different percentile levels are shown in Tables VIII and IX.

None of the groups with suspected osteoporosis, fractured neck of femur and gastrectomy, or the female patients with hypoparathyroidism differ significantly from the normal population. Surprisingly, two-thirds of the male patients with steatorrhoea have better than average densities. This is quite different from the distribution in the 18 female patients with steatorrhoea of whom 83% are below the mean for their age. With regard to the patients with osteomalacia,

all three male subjects and 87.6% of the female subjects are below the 50 percentile level. Two-thirds of the patients (both male and female) with hyperparathyroidism are below the 50 percentile level.

The relation between the mineral in the metacarpal and the spine

The relation between the measurements on the spine and the S.A.E. are considered in Figs 27 and 28. In Fig 27 the percentile levels of the S.A.E. have been plotted against the Spinal Index in 136 female patients referred to a Bone clinic because they were suspected of having osteoporosis. As can be seen, though the spine score of those subjects who are below the 5 percentile level have a slightly lower mineral content than those above this, there are a number of patients with very low spine scores who lie above the 40 and 50 percentile levels. In Fig 28 the spine score is plotted against the actual S.A.E.. If an arbitary line is drawn at a spine score of 80, below which there is definite biconcavity, and an S.A.E. of 28, which is the 5 percentile between 30 and 40 years, it is found that 46% of the patients below and S.A.E. of 28 have low spine scores, and 25% of those above this value have low spine scores.

In Fig 29, the ash weight per cc of the spine has been plotted against ash weight per cc of the metacarpal in 16 unselected patients at post-mortem examination. The values are related ($r = 0.64$; $P < 0.005$).

Conclusions

No firm conclusions can yet be drawn about some of the diagnostic groups as the numbers are too small. However, of the 11 male and female patients with osteomalacia, nearly all have density values (λ) below the 50 percentile. This would be expected, as patients with osteomalacia have osteoid borders which contain virtually no mineral. Of the 29 patients suffering from steatorrhoea, three quarters of them have a S.A.E. below the 50 percentile level and 83% of the female subjects have a low density value. However, two thirds of the 11 male subjects have a density value above the 50 percentile. This may be related to the duration of the disease, 6 out of 8 of the female subjects having suffered from idiopathic steatorrhoea while 7 of the 11 male patients had had gastrectomies less than 10 years before and the steatorrhoea would be of more recent origin. In fact, this suggestion is borne out by the fact that all 4 of the male patients with idiopathic steatorrhoea were below the 50 percentile level.

The low cortical density values obtained in steatorrhoea might be due to early osteomalacia and associated secondary hyperparathyroidism. Alternatively, the greater severity of the osteoporosis may have resulted in an increase in the porosity of the bone which would also lead to a low density. The patients with primary hyperparathyroidism also tend to have a lower than average S.A.E. and cortical density than the normal population. This is compatible with increased bone turnover resulting in a higher percentage of new bone and increased bone porosity. The patients who had undergone gastric surgery show a lower than average whole bone density, 73% of the 89 patients being below the 50 percentile, but their cortical density was not significantly different from the normal subjects, 54.4% being below and 45.6% above the 50 percentile level. This contrasts with the results obtained in the patients with steatorrhoea and osteomalacia, and suggests that these patients are developing osteoporosis rather than osteomalacia. The patients who have undergone gastric surgery are considered in more detail on p. 222. The 6 female subjects with hypoparathyroidism did not differ significantly from the normals in either S.A.E. or λ.

The female subjects who had suffered from fractures of the neck of femur do not differ significantly from the normal population in either whole bone density or cortical density. The male subjects show a normal cortical density but the whole bone density is reduced in 65% of the subjects being below the 50 percentile level. Some caution must be observed as the numbers are relatively small but if this difference between the male and female subjects is genuine then it must be explained. There are several possible explanations: first, that these differences may be explicable by change in the nature of the bone leading to greater susceptibility to fractures in the female subjects: second, that the difference arises from the nature and frequency of the injuries sustained which would require the injuries to be more severe and more frequent in female subjects: third, that fractures occur when a certain proportion of bone loss has occurred, and therefore female subjects who lose a substantial amount of bone will become liable to fracture, whereas only a small proportion of male subjects who fall below this critical value become fracture prone. This is examined further in Chapter III.

The most difficult group to define are the patients with 'osteoporosis'. The female subjects who were referred for investigation because they were thought to have osteoporosis did not in fact differ from the normal population. This is in contrast to the 26 male subjects referred for investigation, of whom 83% were below the 50 percentile level. These differences are certainly due to the way in which the patients were referred to the clinic. The majority of the patients were referred to the clinic because they had attended with a history of back pain or because they had recently had a fracture and on X-ray examination were thought to have 'thin' bones. This assessment is made on an ideal bone thickness judged on the appearance of bones in the young by the referring physician or surgeon or experienced radiologist. It is evident from the inspection of the normal data (Fig 12) that there is a marked fall in the whole bone density of the metacarpal in the normal female subjects, but a lesser fall in the normal male subjects. It would be expected therefore that a greater number of male patients who were judged to have thin bones would be below the 50 percentile simply because normal male subjects show only a

small fall in mineral content with age. The normal female subjects, however, show a marked fall with age, and thin bones, judged on the criterion of bone mineral content in the young patient, will fall within the normal range when age is taken into consideration. However, this merely begs the question as it leaves us no nearer a definition of osteoporosis. The generally accepted definition of this disease (Pommer, 1885; Albright, Burnett, Cope and Parsons, 1941a) is that the amount of bone present per unit anatomical volume is decreased, though the bone that is present is normal both histologically and biochemically. That is, there is thinning of trabeculae, thinning of the cortex and the appearance of increased porosity, but the calcium:nitrogen ratio is normal. That the bone mineral content of osteoporotic bone might be slightly reduced has been suggested by Jowsey and Gershon-Cohen (1964), but further evidence is lacking. Even if there is a small mineral deficiency of 5%, this does not alter the essential problem of definition which is that osteoporosis is simply the result of a decrease in the amount of bone present.

A decrease from a particular level is implicit in this statement, but no attempt has been made to define this level from which the bone mineral content must be reduced for the patient to be considered osteoporotic.

Since the bone mineral content varies throughout life, one possible definition could be to term all subjects who have a mineral content of less than the 5 percentile for their age group as being osteoporotic. However, two important weaknesses emerge. First, it would be possible for a subject who was initially at the 95 percentile to fall to the 10 percentile level, and though this individual will have lost a substantial amount of bone, he will still, by definition, not have osteoporosis. Nor is it possible to know if a measurement is made at a single point in time, if the patient who is at present at the 10 percentile was not always at the 10 percentile or whether he has come down from the 90 to the 10 percentile over a period of some years. Second, this definition takes no account of the fact that for the patient to have a definition of osteoporosis, this must imply that some risk is attached to the level of bone mineral content. For instance, it might imply that the

patient is at risk from the point of view of more easily developing a fracture from relatively minor trauma.

From inspection of Fig. 27 , it is evident that a number of patients with completely normal mineral content for their age have a significant degree of biconcavity of the vertebral bodies which means that there has been crushing of the spine and could reasonably be considered to have osteoporosis. This suggests three possibilities: First, that the S.A.E. is not a good measure of the mineral content of the spine. In fact the post-mortem studies show a good relation between the density (ash per cc) of the spine and the density of the metacarpal ($r = 0.6$; $P < 0.01$). Second, that there is a change in the character of the bone which is more liable to fracture even though the mineral content is relatively normal. This is implied in the electron microscopic studies of Little, Kelly and Courts (1962), but there is little other evidence to support this. In fact, Weaver and Chalmers (1966) have tested the resistance of the spine to a crushing force and found a good direct relation between the force necessary to deform the spine and the

mineral content ($r = 0.827$). Third, if the tendency to fracture is directly related to the mineral content of the spine as implied by the studies of Weaver and Chalmers (1966), then it would be expected that there would be an increased incidence of crush fractures of the spine and peripheral bones as the mineral content of the bone falls. It is evident that the quantitative definition of osteoporosis is a difficult one which must be considered in relation to the incidence of fracture. This is examined in detail in the next Chapter.

In Fig. 28, it can be seen that the 136 female patients referred to the Bone clinic because they were suspected of having osteoporosis show an increase in the incidence of biconcavity of the vertebral bodies with decreased mineral content of the metacarpal. In this Fig. the Spinal Index of 80, shown by the horizontal line, indicates a significant biconcavity. Biconcavity implies crushing and fracture of the trabeculae of the vertebral body. Since the force with which crushing of the spine occurs decreases with decreasing mineral content of the spine and the mineral content of the spine is related to the metacarpal mineral content, then ideally it should be possible to define the possibility of a patient

developing a crush fracture from a knowledge of his S.A.E.. However, biconcavity being a fracture must to some extent depend upon the strength of the force applied at any particular time, and a very large number of subjects would have to be X-rayed to achieve this predictability. Nevertheless, a useful clinical definition of osteoporosis is possible. From inspection of Fig. 26, it can be seen that if an S.A.E. value of 28 is taken as shown by the vertical line, there is a very great increase in the incidence and severity of biconcavity below this value, 46% of the patients having significant biconcavity. The value 28 is the 5 percentile value for normal women between the ages of 30 and 40 years when the mineral content of the bone is at its peak. In the male subjects, the 5 percentile level between the ages of 30 and 40 years when the mineral content of the bone is a value of 27. This definition does serve a useful clinical purpose. First, it is possible to determine the rising incidence of osteoporosis with ageing. This in fact gives a much higher incidence of osteporosis than has previously been described (Collins, 1959; Beck

and Nordin, 1960). If these values are used to define the lower limit of normal, then 68.6% of the female patients referred to the Bone clinic and 40% of the male subjects are below this value. Of the female subjects with fractures of the femoral neck, 84.2% had an S.A.E. of less than 28. Alffram (1964) showed a sharp rise in the incidence of fracture of the femur above the age of 60 years at which age 40% of the normal population have an S.A.E. below 28. Fractures of the lower end of the radius also increase rapidly between the ages of 50 and 60 years of age (Allfram and Bauer, 1962). In the normal population, the number of women who have an S.A.E. below 28 rises from 15% to 40% of the subjects at these ages.

The normal male subjects show a much smaller fall in bone mass with age, and only 11% of the 26 patients referred have an S.A.E. value of less than 27. Of these 26 patients, nine had crush fractures and 6 of these patients had an S.A.E. below 27.

Of the [25] patients with fracture of the neck of the femur,

only 44% had an S.A.E. of less than 27. However, from inspection of Table VII, it can be seen that there is a bimodal distribution in the percentile level of their bone mineral content. This would be expected if the male patients were drawn from two groups, one a younger age group with normal bones and the other a group of patients with minor trauma resulting in fracture because of osteoporosis.

It would appear therefore that the risk of fracture or development of spinal biconcavity and crush fracture of the spine is related to the mineral content of the bone. A useful working definition of osteoporosis is that women with an S.A.E. value of less than 28, and men with a value of less than 27 are substantially at risk in the development of fractures, and should be considered to have osteoporosis. The assessment of the effect of pathological conditions on bone mineral content is best considered in such a way as to allow for the fall in mineral content which occurs with age

in the normal population. This is best done by determining the percentile level which allows for the changes with age. The relation between bone density and fracture is examined further in the following Chapter.

CHAPTER III

THE INCIDENCE OF FRACTURE AND ITS RELATION TO BONE MINERAL LOSS IN MALE AND FEMALE SUBJECTS

Several attempts have been made to relate mineral loss in bone to the incidence of fracture. Newton-John and Morgan (1968) compared the rate of loss of bone with age in women derived from a study of thirty publications, with the incidence of fracture of the neck of femur and the lower end of radius, and were able to demonstrate a good relation between the two.

However, it is evident that there are a number of fractures at a variety of sites which do not fit the pattern described. Such fractures include the phalanges, metacarpals and the upper end of radius. Another important feature to which little attention has been given is the rate of bone loss which is much greater in females than in males and obviously should be related to the incidence of fracture in the sexes. Further, the rate of change in bone mineral is a function of the measurement used (Chap. I). This is associated in part with the changes in external diameter of

the bone to which attention has been drawn by Trotter, Broman and Peterson (1960); Sedlin, Villanueva and Frost (1963); Smith and Walker (1964); Epker and Frost (1966); Takahashi and Frost (1966); Garn, Rohmann, Wagner and Ascoli (1967). It is evident therefore that a more detailed study of the incidence of a variety of fractures in relation to bone mineral content is called for.

Methods and materials

subjects studied

The rates of change in bone mineral content were studied in the volunteers (Groups III and IV) described in Chapters I and II. They consisted of 317 female and 312 male subjects.

The incidence of fracture at different sites in relation to age and sex was obtained from a survey carried out by Knowlden, Buhr and Dunbar (1964). In that study, the incidence of fracture in relation to age and sex is described in 6,500 subjects admitted to hospitals in the Oxford and Dundee areas between 1954 and 1958. The incidence of fracture was calculated using the census publication of

1961 for the two cities. Knowlden et al (1964) chose Oxford and Dundee for their study because the services dealing with fractures were within the well defined city areas and were centralised. This allowed an accurate assessment to be made of the incidence of fracture by site, the relation between the type of accident, and the variation with sex and age.

Bone mineral changes

The changes in bone mineral content with age have been discussed in Chapter I. The rates of change have been calculated from the data on A.E., S.A.E., T.C.M., and S.T.C.M. (Chapters I and II). The densitometric method used is described in Appendix I.

Results

The fall in mineral content for each of the parameters against age was obtained by using the percentile Confidence limits which were calculated for each of the curves. On each of the percentile graphs, a line was drawn parallel to the age axis; the height above the axis

being the maximum value of the 25 percentile curve. The 25 percentile curve was chosen as the most convenient one to use since it fell within the spread of values over the whole age range. The value of prevalence of decreased mineral content at each age is the intercept of the horizontal line on the percentile curves (Fig 30). Prevalence, therefore, is that percentage of the population with values of A.E., S.A.E., T.C.M., and S.T.C.M. which are less than the peak value of the 25 percentile. It is, of course, the rate of chance of prevalence and not the absolute values which are significant in the present context.

As might be expected from an inspection of Figs 17 to 22, the rates of change show a more rapid rise for female subjects than for the males. There is also a marked variation in the rates of change between male and female subjects depending upon the measurement used. In Table X, the difference between male and female bone loss rates are illustrated by taking ratios of the

slopes of prevalence of A_cE_c, $S_cA_cE_c$, $T_cC_cM_c$, and $S_cT_cC_cM_c$ at the age of 50. The prevalence curves are shown in Fig 31. Table XI shows the rate of loss of mineral, using the different parameters of measurement in the male and female subjects between the ages of 35 to 75. The figures are shown as a percentage increase in prevalence of diminished bone mineral using the maximum value of the 25 percentile as the starting value. The rate of increase in total mineral loss ($T_cC_cM_c$) is very low in the male subjects compared with the A_cE_c, $S_cA_cE_c$, or $S_cT_cC_cM_c$. In female subjects, the increase in $T_cC_cM_c$ loss is again the lowest of these parameters, but is more than eight times higher than it is in the male subjects.

Changes in the external diameter of the 3rd metacarpal in relation to age are shown in Fig 19 in the male and female subjects. In the male subjects, there is a small increase of $1\frac{1}{2}\%$ between the ages of 30 and 75 years. In the female subjects, there is a slight increase from age 30 to 55 years of 1.75% which thereafter falls slightly to achieve a value 1% above the starting

value. The changes in medullary width are shown in Fig. 20. The medullary width increases by 33% for the males between 30 and 75 years and by 54% in the female subjects over the same age range. The percentage incidence of fracture against age shown in Fig. 32 which distinguishes between the change in incidence rate for industrial accidents leading to fracture, and the changes in incidence of fractures sustained in the home. When these are compared with the rate of change of fractures at different skeletal sites, certain fractures are found to be sufficiently well reproduced from curve to curve to allow them to be separated into three groups (Figs. 33 to 39) and Table XII. These are:

(A) A group of fractures (Fig. 33) whose incidence tends to diminish with age and which are very similar to the pattern of incidence of industrial accidents shown in Fig. 32.

(B) A group of fractures at other sites (Fig. 34) which show an increasing fracture incidence with age; both males and females being similarly affected. These resemble the age pattern of incidence of fractures occurring in the home (Fig. 32).

(C) A third large group in which the fracture incidence is different from either of the above categories (Figs. 35 to 38 Table XII). The fractures of this group are characterised by an increasing breakage rate with age for females while the males show little change with age up to the age of 75 years.

Most fractures show a marked increase in incidence after the age of 75 years. With a limited number of data points available for fracture rates at each site there is necessarily a certain amount of deviation from the curve which represents a particular group. However, in the majority there is no difficulty in assigning the fracture incidence at a particular site to one of the above groups. Fractures of ribs are exceptional in that they show changes which do not fit into any of the above patterns (Fig 39).

Accidents leading to fractures which occur during industrial activity fall in prevalence after the age of 60 years or more, but account for as many as 45% of the fractures in males in the age group 46 - 55 years. This fall in prevalence is also evident in women (Fig. 32). Fractures due to falls in the home are more prevalent in women, but show a marked

increase over the age of 65 years in both male and female subjects. Over the age of 85 years, 67% of fractures in women and 62% of the fractures in males are associated with falls in the home.

In group (A) there are several obvious sites of fracture which could be expected to occur in an industrial community. These are the phalanges (hands and feet) tarsals, metatarsals, carpals and metacarpals (Fig. 33 Table XII). These sites are at risk in persons involved in manual work and, as might be expected, the incidence rate is greater in the male. The fracture incidence rates do not adhere strictly to the curve representing the incidence of industrial accidents in Fig. 32 partly because of the way in which they are plotted. That is, at all of the sites fracture incidence rates are plotted as fractions of the value of incidence rate at age 40. In this way although information on absolute incidence rates is lost, it is possible to compare the nature of changes in incidence rates with age at all of the sites. However, Group (A) sites are typified by a steady fall in incidence rate after the age of 60 years in the male subjects. There is a very

initial
small rise in the fracture incidence with age at these sites for female subjects.

Examination of the fracture incidence of the pelvis and spine (Fig. 34) shows that these fractures have an increasing incidence with age in both male and female subjects, very similar to the incidence of fracture in the home and these fractures therefore fall into Group (B). Excluded from Group (B) are any fractures showing a marked rise after the age of 75 years in which there is a marked difference in fracture incidence between male and female subjects over the age range 35 to 75 years. These latter are included in Group (C). The main characteristic of this group is the increased fracture incidence with age in female compared with male subjects. There is a tendency for the incidence rate to flatten between 55 and 75 years before taking a marked upward trend after 75 years. In male subjects there is little change in fracture incidence up to the age of 75 years after which there is again a marked increase in incidence rate at many of the sites. The

fractures included in this group are illustrated in Figs. 35 to 38 and listed in Table XII. Fractures of ribs (Fig. 39) are different in the age incidence changes from any of the fractures described above.

Discussion

It is evident that the pattern of change in bone mineral content is very different depending upon the type of measurement used. These differences are important when considering a quantitative definition of osteoporosis and the prediction of the risk of fracture with age.

The various parameters measure different things and account for much of the apparent discrepancies in the literature where bone loss is variously described at ages ranging from 20 years (Epker et al, 1965; Takahashi and Frost, 1966) up to 50 years (Nordin, MacGregor and Smith, 1966; Smith, Anderson, Shimmins, Speirs and Barnett, 1969). It is therefore important to be very clear as to what is measured by each of these parameters as discussed on p. 30. To summarise, A.E. is a measurement of the amount of bone

mineral in a fine column in the middle of the metacarpal (Fig. 1). The S.A.E. measures whole bone density, the T.C.M., the total mineral per unit length of bone and S.T.C.M., the amount of mineral per unit length of bone divided by the square of the external diameter to allow for the difference in size between individuals.

An examination of the factors involved in the calculation of each of the measurements gives the explanation of these differences. The total width of the metacarpal increases by 1.5% in the range 30 - 75 years, but stays almost constant in females, reaching a small peak between 50 and 60 years, and falling slightly thereafter. Sorenson, Mazess, Smith, Clark and Cameron (1968) found an increase of 8% in the width of the mid-shaft of the radius over the same age range in males while the width of the bone remained almost constant. However, Meema and Meema (1969) observed no change in radial width in either male or female subjects, while Garn et al (1967) reported an increase in width of the second metacarpal of

between 2% and 3% for male and female subjects. Epker and Frost (1964) reported a continuing expansion in the diameter of the human rib between 2 and 70 years.

Medullary widths of the third metacarpal were found to increase by 33% in males and 54% in females between 30 and 75 years of age in the present study. There is therefore a redistribution of bone with age with loss from the endosteal surface and deposition on the periosteal surface in male subjects. In the female subjects deposition on the periosteal surface continued up to 55 years of age and fell slightly thereafter. The measurement of the A_oE_o and the $S_oA_oE_o$ involves the absorption of X-rays by bone mineral in one direction only (Fig. 1). Hence, the narrowing of the cortex results in a marked diminution of the A_oE_o and $S_oA_oE_o$ with age since redistribution of mineral within the bone is not allowed for in these estimations. The calculation of the $T_oC_oM_o$ on the other hand involves the use of cortical area so that redistribution of bone is taken into account. The changes in $T_oC_oM_o$ are then attributed to actual changes

in the amount of mineral present per unit length of bone. $S_eT_eC_eM_e$ is calculated by dividing $T_eC_eM_e$ by the square of the diameter. Since bone diameters in males were found to increase with age, then the marked decrease in $S_eT_eC_eM_e$ with age compared with $T_eC_eM_e$ results.

The changes in total cortical mineral described in this study show a similar distribution to the mineral changes described by Sorenson et al (1968) who used a direct scanning absorption technique with a radioisotope as a source of radiation. Using this technique the measurement derived is essentially the same as the $T_eC_eM_e$ described in the present study. They found a fall of about 8% for males and 39% for females over the age range 35 to 75 years. In the same age range, the $T_eC_eM_e$ of the metacarpal in the present study, fell by 4% in male subjects and by 26% in the females.

If the incidence of fractures is to be related to mineral content, then for a mineral parameter, say X, the changes in fracture incidence will be related to the changes in the distribution of the values of X in the population.

That is, changes related to the fraction of the population possessing a value of X which is less than some arbitary fixed value. The value which has been used in this study is the maximum value of the 25 percentile. This fraction, expressed as a percentage, is defined as the prevalence of X.

When the fracture incidence in each of the groups is compared with the curves of prevalence of diminished mineral content (Fig 31), it is obvious that the fractures at the sites of Groups A and B are not related to the mineral prevalence curves. In the case of Group A, where fracture incidence decreases after the age of 50 years in males and females, any relation between mineral content and fracture incidence is probably swamped by the magnitude of the fracture rate associated with industrial accidents. Although the steady increase in fracture incidence for males and females exhibited by sites of Group B (spine and pelvis) might be due to an increased tendency to fall with increasing age, it is not clear why these two

sites should be different in their fracture prevalence from the sites of Group C.

It is at once obvious, when typical fracture incidence curves of Group C are compared with those of prevalence of diminished mineral content, that they are basically similar, with the incidence of fracture and the prevalence of decreased mineral content for females increasing with age much more rapidly than for males. It has in fact been shown by Newton-John and Morgan (1968) that curves representing the prevalence of mineral loss with age for females can be fitted quite well to fracture incidence rate curves for the lower end of radius and the neck of femur. It is these two sites at which the largest difference between male and female fracture incidence is seen. However, since the prevalence of mineral loss increases with age in both male and female subjects, it is evident that any of these parameters may be separately correlated with fracture incidence which also changes with age as has been shown for S.A.E. with increasing biconcavity of the spine in Chapter II. Therefore, when

comparing the prevalence of the various mineral loss measurements with fracture incidence, it is important to take into account the relative behaviour of the incidence rates in both male and female subjects. Where the prevalence of fracture and mineral loss correspond for both males and females, then this is good evidence of relation between these parameters.

The rate of change of loss of bone mineral measured as the tangent to the prevalence curve at the age of 50 years show the prevalence rates for males to females for A_oE_o = 1:2.33, $S_oA_oE_o$ = 1:2.08, $T_oC_oM_o$ = 1:8.33 and for $S_oT_oC_oM_o$ = 1:3.33. Further, while there is a small increase in the prevalence of diminished mineralisation for males with age this is largest for A_oE_o and smallest for $T_oC_oM_o$ (Table XI). The increasing prevalence of mineral loss are shown in Fig. 31 . These curves are very similar to the fracture incidence curves of Group C. Several of the fracture incidence curves can be well fitted by the $T_oC_oM_o$ prevalence curves. These are femur (shaft), clavicle, humerus (upper end),

patella, radius (lower end) and tibia. Several of the other fracture sites show a recognisably similar prevalence pattern, but the fit of the curves is qualitative rather than quantitative. Part, if not the whole, of the explanation here must lie in the limited number of data points because of relatively small numbers.

It might be argued that the prevalence of diminishing mineralisation is derived from measurements of the metacarpal while incidence data involves a variety of sites, hence the two are not strictly comparable. Surprisingly little work has been done in comparing the variation in mineral content of bone with age at a variety of sites. There is a relation between the mineral content of the metacarpal and the spine (Fig 29, p. 39). Furthermore, the incidence of fracture at a variety of sites for both trabecular bone (e.g. lower end of radius) and in many of the sites involving cortical bone agree well with bone mineral loss as measured by the T.C.M. in the metacarpal, which strongly suggests that the rate of mineral loss is similar at these sites.

One further fact calls for comment. It is evident from the inspection of the data in Fig. 31 that while the rate of fall in T.C.M. diminishes with age in both male and female subjects after the age of 75 years, there is a marked rise in the incidence of fracture between 75 and 80 years in both sexes. It is evident that some factor other than the change in mineral content must be evoked to account for this. One obvious suggestion is that there might be an increased tendency to fall awkwardly with age. However, this explanation will not hold for both the steady increase in incidence of fractures in both males and females of spine and pelvis, and the sudden sharp increase in incidence of fractures in male and female subjects after the age of 75 at some of the sites in Group C. Ageing has other effects beside the loss of bone tissue. Jowsey (1963b) showed that there is an increasing proportion of lacunae plugged with calcium phosphate following cell death with increasing age. This might well affect the chemical nature of

the collagen and the nature of the mineral phase. Chatterji and Jeffrey (1968) have shown an increase in the length of the apatite crystals with age which they suggest would lead to increased 'brittleness' of the bone. A possible explanation therefore is that such changes could affect mechanical strength to become the dominant factors determining the incidence of fracture about 75 years of age.

It is evident, however, that if changes in the total cortical mineral (T.C.M.) were used to quantitatively define osteoporosis then all small individuals would be osteoporotic when compared to large and this is not valid. Therefore, though the rates of change in total cortical mineral are clearly best related to the incidence of fracture, the fracture incidence cannot be the factor which determines the quantitative definition of osteoporosis. For this purpose it is evidently necessary to apply a correction for size and this can be done by making use of the width of the bone to derive the S.A.E. which measures whole bone density.

Clearly trabecular bone loss can also be best described by a whole bone density measurement which allows for comparison between individuals. An alternative method would be to use the $S_oT_oC_oM_o$. The rates of change of bone loss here are similar though not identical with that shown by the $S_oA_oE_o$ or whole bone density measurement. There is no obvious reason for choosing one above the other. The measurement which is finally used will almost certainly depend most upon the way the measurements are made. With an x-ray technique, the simplest and most reproducible technique is the $S_oA_oE_o$ measurement and is the one chosen for this purpose in the thesis. The alternative method (i.e. the $S_oT_oC_oM_o$) would be the method of choice if the total calcium content per unit length was measured directly. This is most accurately done using the technique of Sorenson et al (1968).

In conclusion, therefore, the risk of fracture is best defined by measuring the mineral content per unit length of bone. Osteoporosis, however, is best defined in terms of a measurement, such as the whole bone density, which allows for differences in the size of individuals.

CHAPTER IV

THE MEASUREMENT OF CALCIUM ABSORPTION

Many attempts have been made to measure calcium absorption using radioactive calcium as a tracer. Bronner, Saville, Nicholas, Cobb and Wilson (1962) used a double isotope technique, one being given orally and the other intravenously; they measured the absorption of calcium from the differential secretion of the two isotopes in the faeces. A similar study was done by de Grazia, Ivanovich, Fellows and Rich (1965), who measured the differential output in the urine. The relatively simple technique of administering an oral dose of a calcium isotope in 20 to 250 mg of calcium chloride as carrier, and then two hours later measuring the isotope level in the blood has been used by a number of authors (Bhandarkar, Bluhm, MacGregor and Nordin, 1961; Avioli, McDonald, Singer and Henneman, 1965). Jaworski, Brown, Fedoruk and Seitz (1963) administered a single isotope by mouth and measured the loss in the faeces. Nordin, Young, Oxby and Bulusu (1968) extended the idea by including in their calculation equations which measured the rate of disappearance

of isotope from the blood on the assumption that this occurred at a constant rate.

Tests used to measure calcium absorption may be designed for different reasons. Ideally, any method used should measure precisely the function it is designed to test. However, such an ideal may not be achievable or, if it can be achieved, is too complex to be used in clinical practice. This may be a problem especially when large numbers of subjects are being studied. In order to measure calcium absorption in a large population, the method used in the studies described later is that described by Bhandarkar et al (1961). This technique is relatively simple and can be used for large numbers. However, it was necessary to test the efficiency of the method as a measure of calcium absorption. To do this, a double isotope technique has been used to determine calcium absorption, and the result compared with the two hour blood level. An analogue has been used to determine calcium absorption.

Theoretical considerations

The measurement of calcium absorption is a complex procedure for a number of reasons. Fig. 40 illustrates the pathways that an orally administered isotope of calcium may follow once it has been absorbed. After the isotope is ingested, part will be absorbed and part will continue to pass down the gut. Once absorbed the isotope will be distributed throughout a rapidly exchanging pool consisting of the extracellular fluid, soft tissue and some surface bone. Once within this pool, isotope may return to the gut as endogenous secretion, and some of this may be re-absorbed. Some isotope will be lost permanently in the urine. Isotope from the rapidly exchanging pool will also enter the bone by long term exchange and active bone formation. It may return from bone by exchange and a very small amount by resorption (Shimmins and Smith, 1966). In the present study, in order to allow for the changes in blood level due to the

redistribution and loss of the isotope illustrated in Fig. 40 , a double isotope technique has been used. An intravenous isotope (^{45}Ca) has been used to measure the rate of disappearance from the blood pool, and a second isotope (^{47}Ca) has been used to measure the appearance of simultaneously administered oral isotope in the blood. An analogue computer has been used to solve the equations describing this situation.

Procedure

All the patients involved in this study were admitted to a metabolic ward for calcium balance studies over a period of several weeks.

The eleven patients whose results are presented in this study represent a number of clinical conditions, including osteoporosis, hyperparathyroidism, osteomalacia and gastrectomy.

At the time of starting the test the patients had been fasting overnight and their fast continued until two hours after the administration of the isotope. Five μCi

of ^{45}Ca were given intravenously at the same time as the patient drank 20 μCi of ^{47}Ca mixed with stable calcium chloride carrier. Blood samples were removed at 10 minutes, 30 minutes, 1 hour, 2 hours and usually twice more within the first 6 hours. The specific activity of each isotope was estimated as described on p. 153. The analogue computer analysis described below applies to the serum activity within the 6 hour period.

Analysis of results

In analysing the results, it is assumed that equilibrium in the blood pool is reached rapidly and that the pools are of constant size. The actual size of the pools, of course, do not matter. In this circumstance the specific activity can be used as a measure of total isotope in the pool.

The experimental points are plotted out and the ^{45}Ca curve extrapolated back to zero time. This gives the value of the initial voltage used in simulating the behaviour of

intravenously administered isotope. The model further assumes that the movement of endogenous calcium into the gut is negligible within the 6 hour period of this study.

Fig. 41 shows the fractional turnover rates which are evaluated. Ka is the fraction of calcium in the gut transferred to the blood per hour. The analogue model and the pool systems it represents is shown in Fig. 42. The first stage of the analysis is to fit a curve to the data for the intravenous isotope. This is done by applying an initial voltage to integrator number one and adjusting potentiometers 1, 2 and 3. Note that there is no transfer into pool number 3. The kinetic parameters for this two-compartment system have no physiological significance. They do, however, provide an electrical circuit which dissipates charge in the same way as the blood pool dissipates intravenously administered isotope.

The third integrator is now introduced to represent activity in the gut. The initial voltage is transferred to this integrator to represent the orally administered isotope. It is assumed that when this isotope is transferred into the serum pool, its behaviour is exactly that of the intravenously administered ^{45}Ca. The transfer rate into the serum pool is simulated by potentiometer 5 while potentiometer 6 represents other transfer from this gut pool, i.e. movement down the gut. Adjustment of these last two potentiometers makes it possible to obtain a curve which fits the ^{47}Ca serum activity. A typical example is shown in Fig. 43 .

The values found for ka ranged from 0.4/hour to 0.89/hour. That is, on average 68% of the calcium in the first gut pool is transferred to blood in one hour. The values of kg ranged from 0.0024/hour to 0.33/hour. Kg is the fraction of the calcium in this first gut pool which is

removed from the pool by movement down the gut. The values of ka were plotted against the two hour blood levels multiplied by body weight. This plot is shown in Fig 44. Although the relationship is good, the correlation is not ideal ($r = 0.48$). Clearly, the 2 hour blood level is related to parameter ka. Fig 45 shows a plot of ka against the dietary intake less the faecal excretion over a period of two weeks during which our experiment was performed. There is no relationship between ka and the net calcium absorption (diet-faecal excretion) over this period ($r = 0.09$).

No attempt is made to analyse the serum activity fall of radioactive calcium after intravenous injection. This depends on transfer in and out of a number of body pools. This rate of fall is simply reproduced by means of an analogue electric circuit. This makes it possible to obtain a measure of absorption, ka, valid over the 6 hour period of our study. The only assumption being that after calcium is absorbed, it behaves in the same way as the calcium already in blood.

It is evident therefore that this test does not measure calcium absorption in absolute terms. It is possible for instance that even if ka is low, the calcium absorption may still be quite satisfactory, as ka measures the absorption from the upper part of the small intestine. However, ka represents an accurate estimate of calcium absorption from a specific part of the gut, and this does correlate with the 2 hour plasma level which is a practical procedure for measuring absorption in large numbers of patients. That this does not represent the best estimate of net calcium absorption under true physiological conditions is not unexpected, and is probably true of all other isotope tests of calcium absorption.

CHAPTER V

FACTORS GOVERNING THE URINARY LOSS OF CALCIUM

It is evident that changes in the total skeletal mass are governed by the relative rates of bone formation and resorption. Theoretically, therefore, it is possible to determine whether the bone mass is increasing or decreasing at any time by measuring calcium balance. Calcium balance itself is a function of intake and loss from the body. Urinary losses of calcium play an important part in calcium balance. This chapter is devoted to a study of the various factors influencing urinary calcium losses. These are discussed under the following headings:

1) The effect of calcium intake on urinary calcium excretion.

2) The effect of phosphate intake on total and ultrafiltrable plasma calcium, phosphate clearance and urinary calcium excretion.

3) Renal clearance and resorption of calcium in osteoporosis, hyperparathyroidism and hypoparathyroidism.

1) The effect of calcium intake on urinary calcium excretion

The plasma calcium remains remarkably constant despite wide variations in dietary intake (Smith, Davis and Fourman, 1960; Nordin, 1961) though the urinary calcium is known to be influenced by dietary intake. However, it is also known that wide variations in urinary calcium will be found between different individuals on the same dietary intake of calcium. It is clear that the urinary calcium is a function of the filtered load which is in turn a function of the glomerular filtration rate and the level of the plasma water calcium. The level of the plasma water calcium will in turn be influenced by the level of activity of the parathyroid glands, the calcitonin producing cells and vitamin D. The dietary levels of phosphate and sodium may also affect urinary calcium excretion. Since the filtered load of calcium is about 7 gm/day and the urinary calcium 200 mg/day, small changes in the filtered load could account for large changes in the urinary calcium excretion. The following work was planned to test the possibility that changes in urinary calcium might be brought about by small changes in the plasma calcium.

Methods

Subjects studied

Fifty-eight subjects were studied: 11 were members of the staff and 47 were in-patients. Of the 47 in-patients, 11 were suffering from osteoporosis, one from osteomalacia, and 35 had miscellaneous disorders not connected with calcium metabolism, but agreed to the studies being performed. One patient and three members of the staff were studied twice, making a total of 62 studies in all.

Procedure

In preliminary observations on 11 non-osteoporotic patients without any disorder of calcium metabolism, calcium excretion was determined in three 24-hour collections of urine on the ward diet followed by nine on a low calcium diet containing about 250 mg of calcium and 500 mg of phosphorus.

In the next 40 studies (27 of the subjects having no disorder of calcium metabolism, 24 being patients and three members of the staff) blood samples were taken at 9.30 a.m. and 12.30, 2.30, and 4.30 p.m. on two successive days, making eight samples in all from every subject except two from whom only six samples were taken. Urine was collected on both days

and calcium excretion determined. In these studies the diets were given as follows: in 19 the ward diet was given on the first day and the low calcium diet on the second; in 21, the low calcium diet was given on both days but was supplemented with calcium lactate on the first day (in 17) or the second day (in 4) to make a total calcium intake of about 1,200 mg.

In a final series of studies, 11 members of staff took a low calcium diet for two days and ate 1 gm of calcium as calcium lactate on the first day. Blood was taken from them at 9 a.m. and 12 Noon on each day for determination of the ultrafilterable calcium.

Thus, in 47 of the 51 plasma studies, the regime changed from an average to a low calcium intake and in four from a low to an average level. In 32 of these 51 studies, the only change in the diet was its calcium content.

Biochemical methods

Serum and urine calcium were estimated by an AutoAnalyzer (Technicon Instruments) with the following modifications to the standard technique:

1 The alkaline buffer line has an internal diameter of 0.056 in. (1.46 mm) (yellow) instead of 0.045 in. (1.14 mm) (red), giving a delivery of 1.2 ml/minute instead of 0.8 ml.

2 A 40 foot (12 metre) time delay coil in a water bath at room temperature was introduced before the colorimeter.

3 The working standards were made up in 0.15 M sodium chloride.

4 A range expander (Heathkit resistance box) was placed in series with the negative side of the reference photo cell in the recorder.

5 The calcium-releasing agent (hyamine and acetate buffer) was adjusted to pH 4 instead of pH 5.

6 The ammonium purpurate solution was adjusted to pH 7 by adding diethylamine.

7 Urine dilutions were made with 0.15 M sodium chloride.

Ultrafiltration was performed on fresh serum transferred to cellophane tubing without exposure to the atmosphere and centrifuged at 37°C for one hour at 2,000 g in tubes similar

to those devised by Toribara, Terepka and Dewey (1957). The calcium was measured by the AutoAnalyzer technique described above.

Results

The effect of the low calcium diet on the 24-hour urine calcium in 11 subjects is shown in Fig. 46. The mean calcium excretion fell from 243.2 mg on the third day of the ward diet to 179.1 mg on the first day of the low calcium diet. There was little further fall in the next 9 days.

The results of 27 blood and urine studies in patients without disorders of calcium metabolism are shown in Table XIII and XIV. The mean plasma calcium on the ward diet was 9.46 mg/100 ml and on the low calcium diet 9.29 mg. The difference, although small, is significant. The mean urinary calcium excretion in these 27 subjects was 198.8 mg on the normal calcium intake and 135.7 mg on the low calcium intake. The difference of 63.1 mg is significant (Table XIV), P being less than 0.02. The mean calcium/creatinine ratios were 0.17 and 0.12 respectively, and this difference is also significant (Table XV), P being less than 0.02.

The ultrafilterable calcium concentrations in 11 members of staff on the normal and low calcium intakes are shown in Fig. 47. The mean calcium concentration on the normal intake was 5.56 mg/100 ml and on the low intake 5.23 mg. The difference is highly significant ($t = 3.9$; $P < 0.001$). Fig. 48 shows part of the AutoAnalyzer tracing obtained when these ultrafilterable calcium concentrations were determined. The samples obtained on the two diets were fed in alternately and the difference in peak heights is clearly visible.

In considering the relation between plasma and urine calcium the data from all the subjects (including cases of osteoporosis and osteomalacia) have been included. There was no correlation between the absolute plasma calcium concentration and the absolute calcium excretion, but there was a significant correlation between the change in plasma calcium and the change in urine calcium ($r = 0.46$; $P < 0.005$) (Fig. 49). There was a slightly better correlation between the change in plasma calcium and the change in urinary calcium:creatinine ratio (Table XVI).

Discussion

There is a small but significant difference between the mean plasma calcium concentrations in 27 subjects on normal and low calcium diets. Though very small, this difference is more than enough to account for the effect of the change in dietary intake on urinary calcium. In fact, the relation between the plasma and urinary calcium changes is such as to suggest that the tubular maximum reabsorptive capacity for calcium (if such a maximum exists) was not exceeded in this group of subjects as a whole. Thus if one assumes that the ultrafilterable calcium is 53% of the total plasma calcium (Walser, 1961), then the observed mean change of 0.18 mg/100 ml in calcium corresponds to a change of 0.1 mg per 100 ml in the ultrafilterable calcium concentration. At a glomerular filtration rate of 100 ml/minute this could account for a change in urinary calcium excretion of 144 mg/day if tubular reabsorption remained constant (Fig. 49), compared with the observed change of 63.1 mg (Table XIV).

In order to reduce the possibility of error to a minimum most of the samples were put through the AutoAnalyzer in batches of eight, and in many the plasma or ultrafiltrate samples from the two days of the study were fed in alternately. The difference in the heights of the peaks on the normal and low calcium diet days was clearly visible, as shown in Fig 48.

It is not possible to say whether it is the ionic or complexed fraction of the calcium which is primarily involved in the changes observed, nor whether these changes have any effect upon the parathyroid glands. Nordin (1960; 1961) reviewed the evidence which suggests that low calcium diets do not stimulate the parathyroid glands whereas high phosphate diets do, but the two procedures produce an almost identical fall in plasma calcium (Smith and Nordin, 1964). High phosphate feeding produces a rise in the phosphate excretion index (P.E.I.) which is suppressible by calcium infusion, and this strongly suggests parathyroid stimulation (Smith and Nordin, 1964). Low calcium diets, however, do not have any effect on the P.E.I. (Fig 52). Furthermore, Stoerk and Carnes (1945) have shown that high phosphate diets cause an

increase in parathyroid weight in animals. Crawford, Gribetz, Diner, Hurst and Castleman (1957) have shown, on the other hand, that low calcium diets do not cause parathyroid hyperplasia so long as the diets are not deficient in vitamin D. Why comparable changes in plasma calcium should apparently have different effects upon the parathyroid glands is far from clear, but it could be that the calcium fraction involved is different in the two situations or that the assessment of parathyroid gland activity is inadequate.

2) The effect of phosphate intake on total and ultrafiltrable plasma calcium, phosphate clearance and urinary calcium excretion

The parathyroid hormone has at least two actions: a calcaemic effect on bone and a phosphaturic effect on the kidneys. Its secretion is certainly regulated by the concentration of ionic calcium in extracellular fluid, but appears also to be influenced by the inorganic phosphorus concentration. It has long been known that parathyroid hyperplasia is associated with the phosphate retention of renal failure (Bergstrand, 1921), and it has been shown that high phosphorus feeding increases parathyroid weight in animals (Stoerk and Carnes, 1945). However, Ham, Littner, Drake, Robertson and Tisdall (1940) showed that hyperphosphataemia in the absence of hypocalcaemia did not cause parathyroid hypertrophy and it seems probable that the effect of elevated plasma phosphorus on the parathyroids is produced by reciprocal depression of the ionic calcium (Nordin, 1962b).

It has been shown by Goldman and Bassett (1958) that high phosphorus feeding is associated with a rise in the plasma concentration and filtered load of phosphorus, but with a fall in the proportion of the filtered load reabsorbed by the tubules which they attribute to increased parathyroid activity. Somewhat similar results had previously been obtained by Crawford, Osborne, Talbot, Terry and Morrill (1950). Nordin and Fraser (1960) and Nordin (1962) recalculated the data from both these studies and found that the oral administration of phosphorus was associated with a rise in the Phosphate Excretion Index (P.E.I.), which suggested increased parathyroid activity.

The objects of the present study were four-fold: first, to establish whether the raised P.E.I. known to be produced by high phosphorus feeding could be lowered by intravenous infusion of calcium, which would suggest that it was due to secondary hyperparathyroidism (Nordin and Fraser, 1954). Second, to establish by frequent blood

sampling whether the supposed effects of the high phosphorus diet on the parathyroids could be attributed to reciprocal depression of plasma or ultrafilterable calcium. Third the effect on urinary calcium excretion. Fourth, to test the effect of a low calcium diet on phosphate excretion.

Methods

Procedure

There were four series of observations:

1 In 8 patients with osteoporosis the plasma calcium and phosphorus and P.E.I. were determined on the ward diet and after 7-10 days on a dietary supplement of 1.5 g phosphorus (as neutral sodium phosphate). On the following day, the P.E.I. was determined immediately before an 8 hour infusion of calcium gluconate (30 mg of calcium/Kg) and again 18 hours later. The phosphate supplement was administered throughout.

2 In 8 normal subjects, the P.E.I. was measured while on a ward diet and 7 days after starting on a low calcium diet, between 280 and 450 mg calcium per day.

3 In 3 members of staff and 7 patients without metabolic bone disease, plasma calcium and phosphorus were determined at 9 a.m., 12 Noon, 3 p.m. and 6 p.m. on the day immediately preceding, and on the first day of the high phosphorus diet.

4 In 9 members of staff, total and ultrafilterable calcium were measured on samples taken at the same time on the day preceding and on the first day of high phosphate feeding.

Analytical methods: All blood samples were taken with disposable needles and syringes with a minimum of stasis. Blood for ultrafiltration was collected uner paraffin. Serum was separated without delay.

Phosphorus and creatinine were measured in plasma and urine by standard AutoAnalyzer techniques (Technicon Instruments Limited). Calcium was estimated by a modified AutoAnalyzer technique. It is a spectrophotometric method using ammonium purpurate as the indicator. In series 3, calcium was also estimated by titration with EDTA in an EEL titrimeter at pH 10.0 using ammonium purpurate as the indicator. Ultrafiltration was performed on fresh serum transferred to cellophane tubing without exposure to the atmosphere and centrifuged at 37°C for one hour at 2,000 g in tubes similar to those devised by Toribara et al (1957).

Results

First series: the effects of the high phosphorus diet on the plasma concentrations of phosphorus and calcium are shown in Table XVII. In thirty observations on eight patients on the ward diet, the mean plasma concentration of inorganic phosphorus was 3.51 and of calcium 9.62 mg/100 ml. In 29 observations on the high phosphorus diet, the mean plasma phosphorus was 3.92 and calcium 9.54 mg/100 ml. The rise in plasma phosphorus (0.41 mg/100 ml) is significant, but the fall in plasma calcium (0.08 mg/100 ml) is not.

There was a rise in the ratio of phosphorus to creatinine in the urine, the phosphate/creatinine clearance ratio (Cp/Ccr) and the Phosphate Excretion Index (P.E.I.) (Fig 50, Table XVIII). The P.E.I. rose above + 0.09 in all but one case.

The effect of calcium infusions on phosphate excretion in these eight subjects is shown in Fig 51 and Table XIX. The infusions were invariably followed by a substantial fall in the urinary phosphate/creatinine ratio, in the Cp/Ccr and in the P.E.I., the last, in all cases but two, to a subnormal value.

Second series: a low calcium diet had no effect on phosphate excretion (Fig 52).

Third series: multiple blood sampling in ten subjects on the day preceding and the first day of the high phosphate diet yielded the results shown in Fig 53 and Table XX. The mean plasma phosphorus rose from 3.40 mg/100 ml on the day before to 4.16 on the first day of high phosphate feeding: the mean plasma calcium fell from 9.75 to 9.51 mg/100 ml. Both changes were significant.

Fourth series: the total and ultrafilterable calcium in nine subjects on the day before and the first day of high phosphate feeding are shown in Table XXI. The mean total calcium fell by 0.23 mg% and the mean ultrafilterable calcium by 0.29 mg%. Both changes were significant. The estimations were performed in 3 groups, and on each occasion the control and high phosphorus plasma were fed into the machine alternately. A fall in the calcium peak was observed in 7 out of 9 pairs of tracings.

The total and ultrafilterable calcium were also measured by EDTA titration in these nine subjects. As Table XXI shows the EDTA values were slightly higher and more variable than those obtained with the AutoAnalyzer, but the calcium fall in response to phosphate loading was of the same order of magnitude.

The urinary calcium/creatinine ratio was estimated on the first series of eight osteoporotic patients on three days preceding and three days following the start of the high phosphate diet. The mean calcium creatinine ratio fell by 0.10. This fall was highly significant (Table XXII).

Discussion

The results confirm the observations of other workers that a high phosphorus diet not only raises the concentration of inorganic phosphorus in plasma but also produces a disproportionate rise in the Cp/C_{cr} which leads to a rise in the P.E.I. (Goldman and Bassett, 1958; Crawford et al, 1950). The circumstantial evidence that the P.E.I. reflects parathyroid activity is very strong (Nordin and Fraser, 1960; Nordin, 1962) and these observations therefore suggest that phosphorus feeding stimulates the parathyroid glands. This is compatible with the fact that high phosphorus feeding produces parathyroid hyperplasia in animals (Stoerk and Carnes, 1945). It is well recognised that calcium infusions suppress parathyroid activity (Howard, Hopkins and Connor, 1953;

Kyle, Schaaf and Erdman, 1954), and in particular that the high Cp/C_{cr} of osteomalacia can be reduced in this way (Nordin and Fraser, 1954). The present study has shown that the high P.E.I. produced by phosphate feeding can also be suppressed by intravenous calcium, suggesting that it too is attributable to secondary hyperparathyroidism.

There are at least two ways in which phosphate supplements might stimulate the parathyroid glands. The raised plasma phosphorus might act directly on the glands, or they might be stimulated by a 'reciprocal' depression of ionic calcium. There is no evidence that parathyroid activity is governed directly by the concentration of inorganic phosphate in extracellular fluid, but a great deal of evidence that it is governed by the concentration of calcium (Munson, Hirsch and Tashjian, 1963). It is, therefore, inherently probable that the effect of high phosphate feeding upon parathyroid activity is produced by depression of the concentration of calcium.

It proved at first impossible to demonstrate a significant change in total plasma calcium by single daily blood sampling, but the examination of four blood samples a day in ten subjects before and during high phosphorus feeding revealed a small, but highly significant depression of calcium concentration. Any possibility that this might be an artefact produced, for instance, by fluid retention on the high salt load, was excluded by ultrafiltration of serum in nine subjects. This procedure not only confirmed the previous observations but showed that the ultrafiltrable fraction fell if anything slightly more than the total serum calcium.

The results confirm that high phosphate feeding produces a small but rapid fall in plasma calcium. Since the ultrafilterable calcium falls at least as much as the total calcium, it seems reasonable to conclude that it is primarily the ionic calcium which is depressed, and that this is the cause of the parathyroid stimulation which we believe to be demonstrated. The high phosphate feeding also produced an immediate fall in urine calcium. It is

not possible to say at present whether this is simply due to a fall in the filtered load of calcium or whether it too is the result of enhanced parathyroid activity (Kleeman, Bernstein, Rockney, Dowling and Maxwell, 1961).

The effects of phosphate feeding on plasma calcium and $P_{o}E_{o}I_{o}$ which are described are similar to the effects of phosphate infusions observed by Anderson and Parsons (Personal communication). They have found that phosphate infusions produce an immediate fall in plasma calcium and that after a few hours the phosphate Tm begins to fall. The latter observation appears to be analogous to the rise in $P_{o}E_{o}I_{o}$ that has been described and seems to be probably due to parathyroid stimulation, although Bartter (1961) has claimed that it occurs in the absence of the parathyroids.

It is, of course, a well-known clinical fact that a high phosphorus intake is harmful or even dangerous in patients with hypoparathyroidism in whom tetany is easily precipitated. Similarly, the tetany produced in infancy by cow's milk is believed to be due to the high phosphorus content of the latter (Gardner, MacLachlan, Pick, Terry and Butler, 1950). These clinical observations support the

effect of phosphorus feeding upon plasma calcium concentration which have been demonstrated.

It would appear that high phosphate feeding elevates the concentration of inorganic phosphorus in the extracellular fluid, and that this depresses the calcium concentration. The result is a state of secondary hyperparathyroidism and a disproportionate fall in the tubular reabsorption of phosphate which is reflected in a raised P.E.I.. The mechanism of the 'reciprocal' depression of calcium by phosphate is uncertain. It could be due to complexing of calcium by phosphate (Walser, 1961), but since it was also observed with EDTA titration it could also be explained in terms of a physico-chemical equilibrium between bone mineral and its ions in extracellular fluid (MacGregor and Nordin, 1960).

It is evident that the urinary calcium falls with a high phosphorus intake. This could be partially due to a fall in the absorption of calcium due to the formation of calcium phosphate complexes. However, a significant contribution to this fall must be due to the fall in ultrafilterable calcium which in turn leads to a fall in the

filtered load. Another cause of the fall in urinary output of calcium could result from the increased parathyroid activity which causes an increased tubular resorption of filtered calcium, as discussed in part 3 of this chapter.

3) Renal clearance and resorption of calcium in osteoporosis, hyperparathyroidism and hypoparathyroidism

The excretion of calcium in the urine is a function of the filtered load and of the amount of calcium reabsorbed by the renal tubule. Kleeman et al (1958) reported that there was decreased clearance of calcium in normal subjects following the administration of parathyroid hormone during calcium infusion. Loken and Gordan (1959) and Gordan, Loken, Bluhm and Teal (1962) reported that calcium clearance was in fact increased in patients with primary hyperparathyroidism. Bernstein, Kleeman and Maxwell (1963) showed a decreased calcium clearance in patients with hypoparathyroidism given parathyroid hormone during calcium infusion. They also pointed out the importance of associating the calcium clearance with the level of the plasma diffusible calcium. In view of the contradictory findings, the calcium clearance and tubular resorption of calcium has been studied in patients with hyperparathyroidism and hypoparathyroidism, in patients with recurrent renal calculi and in patients with osteoporosis.

Patients studied

The patients in whom calcium clearance was studied all had normal renal function (Table XXIII) as judged by the creatinine clearance. This is important as urinary calcium falls as renal function is impaired (Fig 54). This figure shows the relation between the calcium/creatinine ratios in 15 normal subjects and patients with varying degrees of renal failure.

First, 8 patients with normal renal function were studied. The whole bone density (S.A.E.) was measured in each of the subjects and the percentile value relative to the normal population determined (Table XXIV). Six of the subjects were osteoporotic as defined in Chapter II. That is, they had an S.A.E. value of 28 or under. All were below the 50 percentile value for their age (range 13 to 48), but were well within the two S.D. range defined by the normal population. For convenience, this group is referred to hereafter as the 'osteoporotic' group to distinguish them from the other subjects studied.

Second, nine patients with recurrent renal stone were studied. None of them had evidence of hyperparathyroidism as judged by the P.E.I. (or Cp/C_{cr}). Third, eight patients with primary hyperparathyroidism were investigated. Fourth, two patients with hypoparathyroidism were studied.

Methods

The calcium:creatinine clearance ratios and the calcium reabsorbed per 100 ml of filtrate were estimated in all the patients. In the patients with osteoporosis, renal stone and in two of the patients with hyperparathyroidism this was followed by an infusion of 10% calcium gluconate in physiological saline. It was considered inappropriate to infuse calcium into the patients with primary hyperparathyroidis who had high initial serum calciums. In all the patients, the calcium was infused in a dose of 3.75 mg/Kg/hour. In two of the patients in the 'osteoporotic group' and in two with hyperparathyroidism, the infusion was over a period of 4 hours. In the remaining six osteoporotic subjects and in the patients with hypoparathyroidism, the infusion was continued for 6 hours.

Urine was collected over hourly periods. Blood for total and ultrafiltrable calcium was collected at the midpoint of the urine collection. In the six remaining patients with hyperparathyroidism in whom it was considered inadvisable to infuse calcium, urine was collected over 2 hourly periods and blood collected at the midpoint of the urine collection. The calcium:creatinine clearance ratio and tubular reabsorption of calcium were estimated on these samples.

Calcium was estimated on the plasma, ultrafiltrate and urine by a modification of the AutoAnalyzer technique (McFadyen, Nordin, Smith, Wayne and Rae, 1965). Creatinine was estimated by the standard AutoAnalyzer technique (Technicon Instruments Limited). Ultrafiltration was performed on fresh serum, transferred to cellophane tubing without exposure to atmosphere at 37°C for one hour at 2,000 g in tubes similar to those devised by Torribara et al (1957).

Results

The calcium:creatinine clearance ratio is seen to be directly related to the plasma ultrafiltrable calcium in all four groups of patients (Figs. 55,56 : Table XXV). It is evident that calcium clearance can only be compared in the different groups between the same levels of plasma ultrafilterable calcium. The calcium reabsorbed per 100 ml of filtrate is also directly related to the plasma filterable calcium and it is again evident that the percentage reabsorption in the different groups can only be contrasted at the same plasma ultrafilterable calcium levels.

In Fig. 55 , it can be seen that the calcium:creatinir clearance ratios in patients with primary hyperparathyroidisn are lower than the clearance ratios in the 'osteoporotic group', the 95% Confidence limits of the data being shown by the cross-hatched areas. The differences between the clearances of ultrafilterable calcium between 6 and 9 mg% are highly significant ($P < 0.001$). In the patients with renal stone disease, the calcium:creatinine clearance ratios

are significantly higher than in the patients with primary hyperparathyroidism ($P < 0.001$) (between UF Ca 6 and 9.5 µg), and those in the 'osteoporotic group' ($P < 0.001$). This is more apparent at the higher levels of plasma ultrafilterable calcium. The calcium:creatinine clearance ratios in the patients with hypoparathyroidism are shown in Fig. 56 . The calcium:creatinine clearance ratios are significantly higher than those found in the osteoporotic group and in the patients with primary hyperparathyroidism, but do not differ from the patients with recurrent renal calculi.

In Fig. 57 , the reabsorption of calcium in mg/100 ml of filtrate is seen to be directly related to the plasma ultrafilterable calcium in patients with 'osteoporosis' and in patients with renal stone disease. The reabsorption of calcium in patients with renal stone disease is significantly lower than in the osteoporotic group and this difference is accentuated at the higher plasma ultrafilterable levels, while it does not reach significance at lower levels. In Fig. 58 , the tubular resorption of

calcium in patients with osteoporosis is compared with the patients with primary hyperparathyroidism. The tubular reabsorption is slightly but significantly higher in the patients with primary hyperparathyroidism. In Figs 59 the reabsorption of calcium per 100 ml of filtrate in patients with hypoparathyroidism is compared with the tubular resorption of calcium in the hyperparathyroid patients. The tubular resorption is decreased in the hypoparathyroid patients. Again it is only evident at the higher levels of plasma ultrafilterable calcium. The reabsorption of calcium per 100 ml of filtrate tends to flatten in the renal stone patients and in the hypoparathyroid subjects. This is not evident in either the osteoporotic or the hyperparathyroid group. Thus, at the plasma ultrafilterable calcium levels which have been achieved, while the patients with hypoparathyroidism and renal stone disease appear to be approaching their maximum tubular resorptive capacity per 100 ml of filtrate, it is evident that this has not been reached in the patients with primary hyperparathyroidism and in the 'osteoporotic' group.

Discussion

The subjects in the 'osteoporotic' group have normal renal function and are all well within the two S.D. range of normal in terms of their whole bone density. It is reasonable therefore to compare the calcium clearance and resorption in these subjects with those individuals in the different diagnostic groups.

The lowered calcium clearance and raised calcium reabsorption in patients with hyperparathyroidism, and the raised calcium clearance and lowered resorption in patients with hypoparathyroidism, would appear to support the evidence of Kleeman et al (1958) and Bernstein et al (1963), and not that of Loken and Gordan (1959) and Gordan et al (1962). This confusion has arisen because Gordan and his colleagues have taken the clearance and resorption values in isolation instead of comparing them within similar plasma ultrafilterable calcium levels. The diminished tubular resorption of calcium in the eight patients with renal stone disease is compatible with a renal tubular defect which could be congenital or acquired.

Conclusions

It is evident that the urinary output of calcium is governed by a number of factors. It is influenced by the dietary calcium and phosphorus, which cause small but significant changes in the plasma ultrafilterable calcium. Parathyroid activity itself can be influenced by dietary phosphate intake. The parathyroid activity will determine the plasma calcium level and so alter the filtered load. Increased parathyroid activity also increases calcium resorption by the renal tubule. Diminished parathyroid activity causes increased calcium clearance. The studies in patients with renal stone disease suggest, in some subjects at least, that there may be a change in tubular function which could lead to increased urinary loss of calcium. The part played by urinary losses of calcium in the development of osteoporosis is therefore a complex function of a large number of different factors.

CHAPTER VI

(PART I)

THE RATES OF BONE FORMATION AND DESTRUCTION

The development of osteoporosis must result from the relation between the rates of bone formation and destruction, the bone formation rate being lower than the rate of bone destruction. Either or both could be raised or lowered, but as long as the rate of destruction exceeds that of formation, the bone mass will diminish. Albright et al (1941a) and Albright, Smith and Richardson (1941b) from a study of osteoporosis in post-menopausal women, and women with Cushing's syndrome, suggested that the primary defect in this disease was a failure in the synthesis of bone matrix. This view was held for many years, but with the introduction of the bone-seeking isotopes - ^{45}Ca, ^{47}Ca and ^{85}Sr, a number of authors were unable to find any significant difference in the rate of 'bone formation' in normal and osteoporotic subjects (Fraser, Harrison and Jones, 1960; Dymling, 1964; Nordin, Smith and Glass, 1964; Lafferty, Spencer and Pearson, 1964; Jowsey, 1966). Nordin (1958; 1960) suggested that osteoporosis might result from

prolonged negative calcium balance, and Jowsey (1966) claimed that the rate of bone formation was normal in osteoporotic subjects but that the bone destruction rate was increased. In this study, she used a technique which is discussed below.

The present chapter is divided into two parts. Part I is an examination of the isotope techniques used to determine the rate of bone mineralisation; Part II is an examination of the ways of measuring the rate of bone destruction.

The measurement of bone formation

Measurement of bone formation rate is a problem which has not yet been completely solved. One technique which has been used is by tetracycline labelling which depends upon the measurement of the area between two linear deposits of tetracycline in bone, two doses of the antibiotic being given separated by a period ranging from 14 to 28 days or more. This measurement is possible because the antibiotic is taken up at the sites of active bone formation, and the lines are detectable by their natural fluorescence in ultra-violet light. The area between the fluorescent lines will give a measure of the appositional growth rate. It can be usefully applied to cortical bone but not to trabecular bone. Another technique is that described by Jowsey (1966). This involves the examination of microradiograph sections of bone under the microscope in order to detect and measure sites of active bone formation and destruction. Bone formation and destruction are surface phenomena and the length of the surfaces undergoing formation and destruction can be measured and related to the whole surface length within

the microscopic field being studied. This technique is applicable to relatively small surface areas and the precise measurement of the lengths of the surfaces undergoing bone formation and destruction is difficult. These techniques have supplied useful information though both require bone biopsy and supply information only at a very localised site, and any one site may not indicate what is happening in other parts of the skeleton. These methods have therefore been useful in the study of bone formation in animals, but have so far not been used widely in the study of bone formation in vivo in man.

In man, the study of bone accretion has been largely confined to the study of the mineral phase using the bone-seeking isotopes ^{45}Ca, ^{47}Ca and ^{85}Sr. These isotopes give a measure of the rate of transfer of calcium from blood to bone, and the term Mineral Transfer Rate (M.T.R.) is used hereafter in preference to the term Bone Formation Rate (B.F.R.). The latter implies that the process of bone formation (i.e. the initial laying down of a matrix followed by the calcification of this matrix) is the process that is measured, when in fact only mineral transfer

is measured using radioisotopes. It is evident that these two processes cannot be distinguished by present techniques. A further complication arises from the very rapid exchange from blood to bone because the exchange is occurring from a small pool (i.e. < 1 gm in the E.C.F.) to the very large bone pool, and the correspondingly slow exchange back from the large bone pool to the small extracellular pool. Yet another problem arises if a significant amount of isotope is returned to the blood by bone resorption and exchange, as the bone mineral transfer rate will be underestimated by an amount depending upon the rate of return of isotope. However, an estimate of the rate of isotope return can be made from our investigations (Smith, Speirs and Shimmins, 1967). We showed that the isotope returning from bone enters the E.C.F. and from here part is lost from the body and part is again taken up by bone. Using the mathematical approximation described by Shimmins, Allison, Smith and Speirs (1967), it is possible to estimate the rate of return of the isotope from bone from a knowledge of the whole body

retention and the measured rate of loss of isotope from the body (Fig. 60). With this knowledge, allowances can be made for the slow exchange rate and the true measurement of mineral transfer rate into bone determined (Shimmins and Smith, 1970). However, this technique requires a knowledge of isotope retention up to 90 days or more, and is not a practical technique for measuring mineral transfer in larger groups of patients. From an examination of the data it was apparent that the method used by Bauer, Carlsson and Lindquist (1957) applied between 7 and 14 days after injection of the isotope gives the most accurate assessment of mineral transfer rate measured by ^{85}Sr and between 5 and 10 days using an isotope of calcium. There is discrimination between calcium and strontium in their transfer across biological membranes, and this has been examined in detail below.

If a number of patients are to be studied, then ^{85}Sr is a convenient isotope to use.

It is a γ-emitter, and whole body retentions may conveniently be determined by using a whole body counter; the half-life of the isotope is 65 days and it is therefore very suitable for the study of patients. Because of this, a number of patients have been studied with ^{85}Sr to determine the M.T.R.

Discrimination between calcium and strontium by biological tissues in man

Discrimination between calcium and strontium in the transport of these ions across biological membranes has been recognised for some time. This discrimination has been shown to occur in transport across the gut (Bailey, Bryant and Loutit, 1960; Spencer, Li, Samachson and Laszlo, 1960; Samachson, 1963; Nordin, Smith, MacGregor and Nisbet, 1964b), in reabsorption in renal tubules (Harrison, Raymond and Tretheway, 1955; MacDonald, Noyes and Lorick, 1957; Barnes, Bishop, Harrison and Sutton, 1961) and in placental transfer (Hodges, MacDonald, Nusbaum, Stearns, Ezmirlian, Spain and McArthur, 1950). The discrimination between calcium and strontium at the bone surface has been investigated in several ways. The transfer of strontium and calcium

tracers into bone powder has been followed (Harrison, Lumsden, Raymond and Sutton, 1959; Boyd, Neuman and Hodge, 1959; Neuman, Bjornerstedt and Mulryan, 1963), and the uptake of these tracers in vivo in animals (Bauer, Carlsson and Lindquist, 1955; Comar, Wasserman and Nold, 1956; Likins, Posner, Kunde and Craven, 1959), and in man (Dow and Stanbury, 1960; Nordin, Bluhm and MacGregor, 1962; Bronner, Aubert, Richelle, Saville, Nicholas and Cobb, 1963) has also been investigated.

It is clear that there is discrimination against the transfer of strontium as compared with calcium across biological membranes, though why it occurs is not understood. Whether discrimination occurs at the site of incorporation into bone has not been clearly established.

Two series of studies in man are reported below: first, the investigation of the effect of different stable calcium intakes on the retention and faecal and urinary excretion of ^{45}Ca and ^{85}Sr during continuous administration of these isotopes. This technique is described by Nordin, Smith and Nisbet (1964a): second, ^{45}Ca and ^{85}Sr was administered intravenously by a single injection, and

the bone mineralisation rate was estimated with both isotopes. The results were combined with those of Dow and Stanbury (1960) to compare their uptake into bone.

Experimental methods

All the patients were studied in a metabolic ward and they were undergoing calcium balance studies as well as isotope investigation. The urine and faeces were collected in 7 day aliquots.

Continuous-feeding studies

^{45}Ca and ^{85}Sr were administered simultaneously twice daily with the morning and evening meals. Doses of 0.05 μCi of ^{45}Ca and 0.25 μCi of ^{85}Sr were given in 2.5 to 5 mg of calcium as $CaCl_2$. Three weeks were allowed for equilibration before estimations were begun (Nordin et al, 1964a). The isotopes and stable calcium were estimated in the 7 day urine and faecal collections; simultaneous plasma and urine samples were collected for the estimation of stable calcium, ^{45}Ca and ^{85}Sr.

The net absorption of each isotope was taken to be the oral dose less the faecal activity averaged over 2-3 weeks. Skeletal retention was taken to be the oral dose less the faecal and urinary activities averaged over the same period. The mineral transfer rate (M.T.R.) with ^{85}Sr as tracer was determined by dividing the skeletal retention of ^{85}Sr by the average plasma ^{85}Sr specific activity. The value with ^{45}Ca as tracer was found by dividing the skeletal retention of ^{45}Ca by the weekly urine ^{45}Ca specific activity.

Intravenous studies

Doses of 5 μCi ^{45}Ca and 30 μCi ^{85}Sr were administered simultaneously. Both isotopes were estimated weekly in the urine and faeces. The plasma specific activities were estimated at 10 and 30 min, 6, 12 and 24 hr, and 2, 3, 5, 7, 10, 12, 14, 21 and 28 days after administration. The M.T.R. value was determined by the calculation of Bauer et al (1957) applied at 7 and 14 days. The plasma and urine stable calcium were estimated by a modification of the AutoAnalyzer technique (McFadyen et al, 1965).

Faecal calcium was estimated in an aliquot of a solution of the ashed sample by titrating against EDTA with ammonium purpurate as an indicator. ^{85}Sr was determined in plasma and in urine by counting 5 ml samples in an automatic well scintillation counter. Faecal ^{85}Sr activity was determined by counting an aliquot of homogenized faeces in a large well scintillation counter. Sufficient ammonium oxalate was added to the plasma and urine samples to precipitate all their calcium as the oxalate. This precipitate was then filtered off on to a disc of glass-fibre filter paper, and counted in a thin-window gas-flow Geiger counter. For each sample, the counts in the Geiger counter due to ^{85}Sr were estimated by counting a standard source of ^{85}Sr, firstly in solution in the well scintillation counter and secondly, as a precipitate in the Geiger counter. The ratio of these standard counts multiplied by the count-rate of the sample in the well scintillation counter gave the count-rate of the ^{85}Sr in the Geiger counter. By subtracting this count-rate from the total count-rate in the Geiger counter, the count-rate due to ^{45}Ca was found. Faecal ^{45}Ca was similarly estimated by precipitating the calcium from an aliquot of the

solution of ashed faeces used to estimate the stable calcium. Each isotope sample was counted for the time taken to record 5,000 counts. Therefore, as the count-rate due to the isotope fell, the standard error of the counts due to the isotope increased. The least value of this standard error was 1.5% and this increased to 7% when the count-rate due to the isotope fell to its lowest value.

Thirty-two patients were given ^{85}Sr by continuous feeding and 19 patients were given ^{45}Ca. Six patients were given both ^{45}Ca and ^{85}Sr by intravenous injection. The clinical details are shown in Tables XXVI, XXVIII. The results of these investigations are shown in Figs 61 to 65 and Tables XXVII, XXVIII. The patients described as having osteoporosis had decreased bone mass, but did not differ significantly from normal subjects when age was taken into consideration. This point has been discussed in detail in Chapters I, II and III.

Results

At all levels of calcium intake in the continuous feeding studies, the net absorption of radiostrontium is less than that of radiocalcium (Fig. 61). If the discrimination against strontium is simple competitive inhibition, it would be expected that the discrimination would increase with increasing calcium intake. However, there is no change in discrimination with increasing calcium intake, the average discrimination value being 0.69. Bailey et al (1960) and Samachson (1963) found a discrimination factor of 0.5. Spencer et al (1960) found a factor varying from 0.40 to 0.45 by a single oral dose of the isotopes. Nordin et al (1964b) found a factor of 0.55 using a single oral dose and measuring the level of ^{45}Ca and ^{85}Sr in the plasma two hours later.

Discrimination by kidney

The urinary excretion of ^{85}Sr and ^{45}Ca during continuous feeding of isotope at different calcium intakes is shown in Fig. 62. The urinary excretion of ^{85}Sr is the same or slightly higher than that of ^{45}Ca at all levels of calcium intake, and the excretion of both

isotopes decrease with increasing intake. Since the renal excretion of ^{45}Ca and ^{85}Sr depends in part upon the plasma levels of these isotopes, discrimination by the kidney can only be shown by comparing simultaneous plasma and urine samples. This has been done and the results are shown in Fig. 63. When compared in this way, the urinary excretion of ^{85}Sr is 3.34 times higher than that of ^{45}Ca. This agrees well with the factor of 3.5 found in six normal subjects by Barnes et al, (1961). Discrimination might arise in the glomerulus during filtration of plasma water or during reabsorption of calcium and strontium by the renal tubule. If the protein binding of strontium is the same as calcium, then the above factor will be determined by tubular reabsorption assuming that all the smaller complexes are filterable. If the binding of strontium is different from that of calcium, then the renal discrimination factor would be affected by filtration. If the protein binding of strontium is less than that of calcium, as has been suggested by Samachson and Lederer (1958), this would itself

tend to cause discrimination in favour of strontium. In order to test whether the protein binding of calcium was different from the protein binding of strontium, serum samples drawn from patients given strontium were divided into two. The serum calcium and ^{85}Sr activity were measured in the first sample. In the second sample, the serum was ultrafiltered by the method described earlier (p. 115) and the serum calcium and ^{85}Sr activity determined in the ultrafiltrate. The specific activities (^{85}Sr/mg calcium) were found not to differ significantly (Fig 64) indicating no significant difference in the serum protein binding of strontium and calcium.

Skeletal discrimination

In the present investigation, the skeletal retention of ^{85}Sr is lower than that of ^{45}Ca at all levels of calcium intake in the patients on continuous feeding of isotopes. The differences between ^{45}Ca and ^{85}Sr are factors of 0.67, 0.37, 0.67 and 0.59 at intakes of 10, 10-20, 20-30 and 30-40 mg/Kg/day (Fig. 65). These figures demonstrate that the proportion of ^{85}Sr retained shows little change compared with ^{45}Ca, with increasing calcium intake. The retention of both isotopes fell with increasing calcium intake. Because of discrimination against strontium at the gut and kidney, skeletal discrimination can only be shown by measuring the mineral transfer rate. The skeletal uptakes of ^{45}Ca and ^{85}Sr with the continuous feeding technique show a higher mineralization rate with ^{85}Sr, but the difference between the two is not significant (TableXXVII). There is no correlation between the ^{45}Ca and ^{85}Sr M.T.R. values possibly because of the difficulty in obtaining an average plasma specific activity for ^{85}Sr as the isotopes were administered twice daily. Because of this difficulty, the method of Bauer et al (1957) was used to calculate M.T.R. between 7 and 14 days, following the simultaneous intravenous administration of ^{45}Ca and

^{85}Sr. This method of calculating M.T.R. assumes that no isotope returns from the bone by resorption of labelled material, and that the plasma has the same specific activity as the rapidly exchanging calcium pools. The M.T.R. values of our six patients are shown in Table XXVIII. The only comparable data are those of Dow and Stanbury (1960), which are in such a form that it is possible to calculate the mineral transfer rates in the same way by using the Bauer bone-mineralisation rate between 7 and 14 days. This has been done and the combined data are shown in Fig. 66. There is a correlation between the measurement of transfer of calcium into bone by using ^{45}Ca and ^{85}Sr as tracers if all the cases are considered ($r = 0.89$) though there are individual discrepancies. Since the M.T.R. values do not have a normal distribution, only the normal and osteoporotic subjects are considered in Fig. 67 and the ^{85}Sr M.T.R. values are significantly lower than the ^{45}Ca values in this group. As it is the ionic calcium and strontium that is transferred into bone, a difference in the rate of transfer of mineral from blood to bone, obtained by using ^{45}Ca and ^{85}Sr as tracers, could be due to the binding of ^{45}Ca being different from the binding of ^{85}Sr. Harrison et al (1955) found no difference between the protein binding of calcium and strontium in two patients, but Samachson and Lederer (1958) added ^{45}Ca

and ^{85}Sr to plasma in vitro and showed that the binding of ^{85}Sr was less than ^{45}Ca. If the calcium and strontium are equally bound then it is permissible to calculate mineral transfer rate into bone by using plasma specific activity of ^{85}Sr and ^{45}Ca. If the binding of strontium is less than the binding of calcium, the estimated bone-mineralisation rates with ^{85}Sr are too high, and the difference we have found between the bone-mineralisation rates measured with ^{45}Ca and ^{85}Sr would be even greater than is shown in Fig. 67. Though the protein binding was not found to differ significantly in the present study (Fig. 64) there are other forms of bound calcium which could affect the results (Walser, 1961). A difference in the transfer rate of strontium and calcium into bone has been described in rabbits by Kshirsagar, Lloyd and Vaughan (1966). They continuously fed young rabbits on a diet containing stable strontium and found that the skeletons of these animals contained a lower Sr/Ca ratio than the plasma and concluded that there is a discrimination factor of about 1.6 in favour of calcium in the transfer from blood to bone. However, this discrimination factor is only valid if the whole skeleton is labelled with strontium and is

in equilibrium with plasma, and this has not been clearly demonstrated to be so in these studies. Other workers have found no difference in the rate of skeletal uptake of these isotopes in animals (Bauer et al, 1955; Comar et al, 1956; MacDonald et al, 1957). In vitro experiments with bone powder in a bathing fluid have shown a discrimination in favour of the transfer of calcium ions into the bone powder in several studies (Harrison et al, 1959; Boyd et al, 1959; Neuman et al, 1963). The uptake of isotope in bone powder is, of course, due to exchange. The discrimination appears to depend upon many factors, such as temperature, pH, ionic concentration of calcium and phosphate, and the concentration of other ions, and there seems to be no good reason why this will not occur in vivo. Boyd et al (1959) in their work on bone powder have postulated that under certain conditions the limiting factor determining exchange of calcium and strontium into bone is the release of stable calcium from the bone. Neuman (1964) also suggested two different lattice positions for calcium ions, one of which is not available for exchange of strontium ions. The evidence thus does support a discrimination against the uptake of ^{85}Sr by bone, but there is a correlation between ^{85}Sr and ^{45}Ca so that relative changes in M.T.R. of calcium may be determined from the study of the skeletal uptake of ^{85}Sr in man.

Bone mineral transfer rates in relation to age

The difficulties and limitations of measuring isotopes have been discussed above. However, there does appear to be a relation between the true bone formation rate and the mineral transfer rate (Heaney, 1964). The problem lies mainly in the amount of mineral transferred from the extracellular fluid by exchange in contrast to the amount taken up by true bone mineral accretion. Heaney (1964) points out that from clinical data high accretion rates as measured by isotope kinetics are found in those pathological states in which high bone formation rates are apparent from the measurement of the alkaline phosphatase which is known to be related to bone formation rate, and in whom the histological features are in keeping such as Paget's disease, hyperparathyroidism, and bone disease associated with hyperthyroidism. Conversely low values are found in those clinical conditions in whom the bone turnover rates are known to be low such as hypothyroidism and hypoparathyroidism.

It is further evident that if the conditions associated with secondary osteoporosis, such as osteoporosis following gastrectomy, and associated with steatorrhoea, Cushing's disease, hyperthyroidism and other conditions with a clear metabolic or endocrine upset are excluded, then osteoporosis is most clearly

linked to ageing (Chapters I and II). The following study therefore is concerned with the measurement of the Mineral Transfer Rate in relation to age.

Method

Patients were given 30 μc of ^{85}Sr by intravenous injection. The whole body retention of the isotope was measured using the whole body counter of Glass et al (1964) modified by adding more shielding round the detector without altering the iso-response field. The patient first lay supine and then prone on an arc of 1.25 m radius. A plastic scintillator of 17.5 cm diameter and 10 cm depth was placed at the centre of the arc. The whole body retention was calculated as the mean of the counts in the supine and prone positions. Whole body retention was measured 2 hours after the injection, and this was taken as the 100 per cent retention value. Counts were repeated every 3rd or 4th day thereafter for 14 to 21 days. Blood samples were taken at the same time as the whole body counts were measured. The specific activities (i.e. per cent dose ^{85}Sr/mg Ca) of the sera from the blood samples were measured by counting 5 ml of sera in a Nuclear Chicago automatic well scintillation counter and estimating the calcium in each serum calcium.

Since discrimination is not possible with a plastic scintillator, and as redistribution of isotope within the body might affect the counts obtained, the whole body retention was examined further. This was done by comparing the whole body retention obtained in the counter with that obtained from estimating and subtracting the isotope lost in the urine and faeces from the dose given. All collections were done in a metabolic unit, and the results are compared in Table XXIX. There was good agreement between the results using these two techniques. The mineral transfer rates were estimated between 7 and 14 days using the calculation of Bauer et al (1957).

Patients studied

The patients studied comprised 65 females referred to a special Bone clinic for further investigation because they were considered to be osteoporotic by the referring physician or surgeon. Each of the patients had a hand X-ray and the metacarpal index (Appendix II) was measured (Table XXX).

Results

The age, weight, mineral transfer rates and metacarpal indices of each of the patients is shown in Table XXX. There was no significant fall in weight with age (Fig 68). There was a fall in the Metacarpal Index (M.I.) with age (Fig 69). This measurement did not differ significantly from the M.I. measured in the 152 normal subjects (Group I - Chapter I). The weight of these latter subjects falls slightly but not significantly with age. The mineral transfer rate measured by the method of Bauer et al (1957) between 7 and 14 days are also shown in Table XXX. The mineral transfer rate fell significantly with age ($P < 0.005$), the rate of fall being 140 mg/day (i.e. 310 to 170 mg/day) between 40 and 90 years of age (Fig 70). This means a fall of:

$$\frac{\left(\log_e \frac{170}{310}\right)}{50} \times 100 = 1.27\% \text{ per year}$$

CHAPTER VI
(PART II)

THE MEASUREMENT OF BONE DESTRUCTION RATES

The measurement of the bone destruction rate in man is a more difficult problem than the measurement of the bone formation rate. At present there is no way of measuring the bone destruction rate directly using bone-seeking isotopes. However, if the bone formation rate is measured at the same time as a calcium balance is performed, then the bone destruction rate can be determined from the difference between them. The one direct method of measuring bone resorption is that described by Jowsey (1966) which has been discussed on p. 145. The problem with this technique is that it is limited to a small area only and it may not be representative of the whole skeleton.

Another method which has been used to determine the relative rate of bone resorption in man is to measure the excretion of hydroxyproline in the urine. Hydroxyproline is an amino acid present exclusively in collagen. Various estimates of the amount of bone collagen in the body range from 40% (Lightfoot and Coolridge, 1948) to 57% (Klein and Curtiss, 1964). The urinary hydroxyproline is not a direct measure of the collagen turnover as some is coming from sources other than bone, such as skin, while some collagen is also reutilised in the body amino acid pool.

However, it has been shown by Gerber et al (1960) using ^{14}C labelled proline, and by Neuberger and Slack (1953) that bone collagen turns over two to five times faster than skin collagen. Therefore, over 70% of the urinary collagen will have come from bone.

Prockop and Sjoerdsmo (1961) considered that the urinary hydroxyproline measured collagen breakdown, and would therefore reflect the rate of bone resorption. High values have been reported in these conditions in which the bone turnover is known to be high, such as Paget's disease, hyperparathyroidism with bone disease and in hyperthyroidism (Dull and Henneman, 1963). Conversely, low values have been reported in conditions in which the bone turnover is known to be low, such as hypoparathyroidism and hypothyroidism (Benoit et al, 1963). Klein and Curtiss (1964) suggested that the urinary hydroxyproline was derived during the presence of bone synthesis. They derived this opinion from the measurement of urinary hydroxyproline and calcium excretion in rats following parathyroid hormone injection. They claimed that a fall occurred in the urinary hydroxyproline at the same time as there was a rise in the urinary calcium followed by a rise

in the urinary hydroxyproline to higher than normal values four days later. They interpreted this as indicating an initial fall in the rate of bone formation, as suggested by the studies of Gaillard (1961), and the subsequent rise in urinary hydroxyproline as being due to a rebound rise in the bone formation rate. However, these findings are in direct contradiction to those of Henneman (1964) who injected parathyroid hormone in man in both normal and hypothyroid patients in whom no initial fall in urinary hydroxyproline excretion occurred. There was a rise in serum and urine calcium from the start. The urinary hydroxyproline rose four days later as occurred in the experiments of Klein and Curtiss (1964). In a patient who had a parathyroidectomy for primary hyperparathyroidism, Henneman (1964) found a rise in the alkaline phosphatase at the same time as there was a fall in the urinary hydroxyproline. In a patient with Paget's disease he demonstrated a fall in the alkaline phosphatase with a rise in the urinary hydroxyproline when the patient was immobilised. Klein and Curtiss (1964) also demonstrated a fall in urinary hydroxyproline and alkaline phosphatase in patients suffering from rickets who were being treated with vitamin D. However, this is contradictory to

the rise in hydroxyproline excretion reported by us (Smith and Nordin, 1964; Nordin and Smith, 1967), when patients with osteomalacia were treated with vitamin D. Further, the dichotomy between the serum alkaline phosphatase and urinary hydroxyproline excretion is well shown in patients with hyperthyroidism in whom values of hydroxyproline excretion may reach more than twice the normal value without a corresponding rise in serum alkaline phosphatase. It is also evident from the values given by Klein and Curtiss (1964) for bone formation and bone destruction that in those conditions where the bone formation is raised, there is also a rise in the rate of bone resorption.

The object of the present study was to determine the bone destruction rates on a varied calcium intake by an isotopic method: to compare the results obtained with the changes in urinary hydroxyproline output in the same subjects: to examine the urinary hydroxyproline output in relation to age: to test the effects of increased calcium intake on the 24 hour urinary output of calcium.

Procedure and methods

Three studies are described below:

In the first study, 14 subjects were investigated. Eleven were osteoporotic as determined by their bone density, the remaining three were normal. Details are shown in Table XXXI.

All the subjects were admitted to a metabolic ward and were maintained on a constant diet. The calcium intake was varied as shown in Table XXXI. A dose of between 0.03 and 0.05 μCi of ^{45}Ca was administered twice daily in 2.5 mg of calcium as calcium chloride. The urine and faeces were collected in 7 day aliquots and stable calcium and ^{45}Ca determined (p.153). In 9 of the osteoporotic subjects, the urinary hydroxyproline was determined on 7 day urinary collections.

In a second study, 24 hour urinary collections were made on 119 female subjects and the urinary hydroxyproline measured. The urinary hydroxyproline output in relation to age was examined. In 106 of these patients, the urinary creatinine was measured on the same 24 hour urine collection, and the urinary hydroxyproline/creatinine ratio plotted against age.

In a third study, a group of 87 subjects were investigated. 24-hour urinary samples were collected and the urinary hydroxyproline was estimated. In 75 of these subjects the urinary collection was made while they were on their home intake. In 64 of these subjects the urinary hydroxyproline was estimated after the addition of calcium supplements (calcium glycerophosphate gm 2, t.i.d.) to their home intake for a period of not less than one week. Fifty of the subjects were common to both groups. All of the urinary hydroxyproline collections were carried out on these subjects while they were on a gluten-free diet.

The stable calcium in urine and plasma was determined by a modification of the AutoAnalyzer technique as described on p. 115. The faeces were ashed and the calcium determined by titration with EDTA using ammonium purpurate as an indicator. Radiocalcium was assayed as described on p. 153. Urinary hydroxyproline was estimated by the method of Prockop and Udenfriend (1960).

Calculations

The bone mineralisation rate was estimated as described on p. 152. The bone resorption rate was estimated by subtracting the calcium balance from the bone mineralisation rate.

Results

The plasma specific activity was recorded at frequent intervals during the first 21 days of isotope administration in 11 patients as shown in Fig 72. The plasma specific activity rose for approximately 10 days and then became stable, but the urinary specific activity appeared to rise for about 2-3 weeks (Fig 73). This stable level was generally lower than the specific activity of the diet, the difference between the two being a function of the calcium intake.

The mean weekly percentages of the dose excreted in the first 8 weeks is shown in Table XXXII and Fig 73. The mean faecal activity varied between 55 and 65% of the dose and did not change significantly with time, although the mean levels were slightly lower during the first two weeks than subsequently. The urinary activity rose slightly in the first 3 weeks to reach what was virtually its equilibrium value of about 10% of the dose by the third week. The mean isotope retention fell to its final value of about 25% by the fourth week.

Effect of intake on excretion and absorption

The relation between calcium intake and isotope excretion and retention are shown in Table XXXI and Figs 74 and 75. There was no relation between calcium intake and the amount of isotope excreted in the urine. Faecal activity, however, rose with increasing calcium intake from approximately 50% to 70% of the dose at the higher intake (Fig 74).

There was a definite fall in isotope retention with increasing intake due to reduced absorption (Fig 75). The absorption of the stable and labelled calcium are related to the calcium intake (Fig 76). The absorption of the labelled calcium falls with intake, the absorption of stable calcium rising with intake.

Bone mineralisation and destruction rates

The mineralisation rates and destruction rates are shown in Table XXXI and in Figs 77 and 78. The bone mineralisation rates ranged from 2.7 to 17.2 mg/Kg/day, but the majority were below 10 mg/Kg/day. The mineralisation rate was independent of the calcium balance as shown in Fig 77. There was an inverse correlation between the calcium balance and the bone destruction rate as shown in Fig 78.

In 9 of the osteoporotic patients the urinary hydroxyproline was estimated during the period of the above study. The urinary hydroxyproline output was inversely related to the calcium balance (Fig 79) and directly related to the bone resorption rate (Fig 80).

In a group of 106 subjects, the urinary hydroxyproline was collected over a period of 24 hours while they were on a home dietary intake from which gelatin was excluded. Eighty-two of these subjects had been referred for investigation of osteoporosis. Their metacarpal indices were measured and the results compared with those obtained in 152 normal women. It is evident from Fig 81 that they did not differ significantly from the normal subjects. The remaining 24 patients did not have their metacarpal indices measured but there was no history suggestive of bone disease, endocrine disease or steatorrhoea. In Fig 82, the 24 hour urinary hydroxyproline was plotted against age. There was a tendency for the hydroxyproline to be higher in the older patient, but this did not reach significance. In Fig 83, the urinary hydroxyproline/creatinine ratio measured in the same urine samples in 106 patients showed a significant rise with age. Fig 84 illustrates the urinary hydroxyproline excretion in the 75 subjects studied on their home intake and in 64 with the addition of calcium glycerophosphate gm 2, t.i.d.. The patients on calcium supplements in addition to their home intake had a significantly lower urinary hydroxyproline output ($P < 0.01$).

Discussion

The measurement of bone formation rate is clearly important in the determination of the cause of loss of bone with advancing age, especially in women. The most practical method of determining the rate of bone formation in man is the use of bone-seeking isotopes. The two most commonly used isotopes are those of calcium and strontium. The early part of this study is concerned with the comparison of the rates of calcium and strontium transfer across the gut, of the comparative rates of loss through the kidney, and the relative rates of uptake by the bone.

There is clearly discrimination against the transport of strontium across the gut as has been shown during the twice daily oral administration of these isotopes over a period of several weeks to patients undergoing a calcium balance study. The average discrimination was 0.69 which compared well with a factor of 0.5 found by Bailey et al (1960) and Samachson (1963), and a factor of 0.55 found by Nordin et al (1964b), the factor in the last three instances being determined by a single oral dose of the two isotopes. A similar but slightly lower factor

ranging from 0.40 to 0.45 was found by Spencer et al (1960). These authors again used a single dose of the isotope. The discriminatory factor of 0.69 in the present series was not found to alter with varying calcium intake, and simple competitive inhibition is therefore unlikely to contribute to this discrimination.

There is also renal discrimination between calcium and strontium as judged by the urinary losses of ^{45}Ca and ^{85}Sr. This results in a loss of strontium in the urine 3.34 times greater than that of calcium. This could be due to lower plasma protein binding of strontium, but from Fig. 64 it can be seen that the protein binding of ^{45}Ca and ^{85}Sr did not differ significantly. The other site of discrimination could be in the reabsorption of these ions by the renal tubule, and would appear to be the likely site at which discrimination occurs.

The skeletal retention of both ^{45}Ca and ^{85}Sr fell with increasing calcium intake. However, as there is discrimination against strontium in its transport across the gut and across the renal tubule, discrimination against strontium in its uptake by the skeleton must be tested for by measuring the mineral

transfer rates. The urinary specific activity of calcium correlates well with the serum specific activity but because of discrimination against the reabsorption of strontium, the urinary strontium specific activity is not representative of serum specific activity. In the continuous feeding experiment, the plasma specific activities vary with time because of the twice daily administration of the isotope. Thus, this is not a satisfactory method of comparing the uptake of calcium and strontium by bone. This is best done by giving a single intravenous injection of both ^{85}Sr and ^{45}Ca and estimating the mineral transfer rate between 7 and 14 days by the method of Bauer et al (1957). When this method is used there is evidence of discrimination against the uptake of strontium compared with calcium in the uptake of these isotopes into bone. Moreover, there are individual discrepancies. However, the M.T.R. measured by strontium does correlate with the measurement using calcium ($r = 0.89$) and it is therefore legitimate to use ^{85}Sr

and though it does not measure the true rate of calcium transfer it does measure a function of it.

Since ^{85}Sr proved a useful isotope in practice, it was used to measure the M.T.R. in 65 subjects who were referred to a special Bone clinic because they were considered to be osteoporotic. As can be seen from Fig 69, these patients do not differ significantly from normal subjects as measured by the Metacarpal Index. As discussed in Chapter I, the total cortical mineral in female subjects falls from 2.5 to 1.8 between 40 and 75 years of age:

$$\frac{\left(\log_e \frac{1.8}{2.5}\right)}{35} \times 100 = 0.94\% \text{ per year}$$

This figure is very close to the rate of fall of the M.T.R., and means that there is no significant fall in the M.T.R. per unit mass of bone with age though there is a fall in the total bone mineral transfer rate per year. The rate of fall in bone mass of a largely trabecular portion of bone mass is 1.15% per year in preliminary measurements done by us using a

photon absorption technique, but this does require to be confirmed.

One of the major problems in any isotope study is to determine the relation between bone formation and the mineral transfer rate. From a study of quantitative autoradiography (Marshall, White and Cohen, 1959a; Marshall, Rowland and Jowsey, 1959b; Marshall, Jowsey and Rowland, 1959c; Marshall Rowland and Jowsey, 1959d; Lacroix, 1962) it is evident that a bone-seeking isotope may enter bone and remain there for a reasonable time. First, forming osteons rapidly take up considerable quantities of isotope. These areas appear as intense black hot spots on the autoradiograph. Second, osteons which are not completely mineralised take up smaller quantities of isotope as they slowly mineralise. They appear as less intense hot spots in autoradiographs. Third, the rest of the bone takes up the tracer uniformly. This is the diffuse label. The uptake of isotope is illustrated in Fig. 71. Any newly formed bone will pass through each of these stages in turn. Further, short-term exchange processes occur which give rise to intense hot spots round blood vessels which disappear

soon after injection. Thus, a proportion of the isotope uptake measured by the M.T.R. will be true bone formation and a proportion will be due to exchange. Rowland (1961) calculated that in dogs given Ra^{226} the fraction of the isotope in diffuse label was 0.43 per cent. In 16 patients who had ingested Ra^{226} the ratio was 0.47, 23-41 years after taking the radium. If this held for ^{85}Sr then the true bone formation rate would be lower than the value given by the mineral transfer rate. There is discrimination between calcium and strontium at the bone surface, the ratio at 7-14 days being 0.85:1. The ^{85}Sr M.T.R. would therefore be about 30% higher than the true bone formation rate. However, of even greater importance is the constancy of the proportion of the isotope going into bone by exchange and true bone formation. This has been examined by us in the dog using quantitative autoradiography (Shimmins, Lee, Wood and Smith, 1970). In this study a comparison was made between the results obtained by autoradiographic studies of the quantity of isotope distributed as diffuse label and hot spots, and the bone formation rate as measured by tetracycline labelling.

Our study was carried out on an elderly dog and compared with detailed information available from a study carried out by Lee, Marshall and Sissons (1965). Three facts emerged from this study. First, the rate of bone mineral transfer measured by ^{45}Ca gave a higher value than that obtained from tetracycline, but the values correlated highly ($r = 0.93$). Second, the bone formation rate measured with tetracycline and the mineral transfer rate measured by ^{45}Ca both fell markedly with age. Third, the amount of isotope in the diffuse label fell with age so that the mineral transfer rate bore a constant relation to the true bone formation rate.

In the dog, the tetracycline value for the true bone formation rate was lower but very close to the mineral transfer rate. Few tetracycline label studies have been done in man, but the tetracycline values that have been obtained suggest a true bone formation rate considerably below that measured by the use of isotopes. This in itself would not matter so long as exchange and formation alter in the same proportions, but further information is required on this point.

Jowsey (1966) using the technique of microradiography to measure bone forming and resorbing surfaces, claimed that the rate of bone formation per unit mass did not fall in osteoporosis but that the rate of bone destruction increased. She did not study the changes in relation to age, but it is evident that osteoporosis, that is loss of bone mass, is a function of ageing, **as shown in Chapters I, II and III.** Dymling (1964) also showed a lower mineral transfer rate in osteoporotic subjects and claimed that if the loss of bone mass with age was allowed for, then there was no significant difference between the normal subjects and the osteoporotic subjects. Both these studies are therefore compatible with the results obtained in the present study, which shows no significant fall in the mineral transfer rate when bone mass has been allowed for.

The measurement of bone resorption rate is a more difficult problem. Values for bone destruction rate can be determined with isotopes by measuring the mineral transfer rate and the calcium balance, and the difference will give the bone destruction rate. The difficulties involved in the measurement of the true bone formation rate have been discussed above.

The measurement of the magnitude of the bone destruction rate will be subject to the same problem, though the tendency here will be to under-estimate the true value. In the present study, the object was to try and establish the relation between the rate of bone destruction and the urinary hydroxyproline output. There has been considerable controversy about this point. It is clear that urinary hydroxyproline output is a measure of bone 'turnover', this term being used to include formation and destruction. The output is high when bone turnover is high as in Paget's disease and in patients with acromegaly (Nordin and Smith, 1965). To establish whether hydroxyproline output measures bone destruction rates or bone formation rates, it would be useful to study any condition in which either the bone formation or bone destruction rates were significantly different from one another. In most conditions where the bone formation is raised there is also a rise in the destruction rate due to remodelling as in Paget's disease and in childhood. Henneman (1964) described a fall in the alkaline phosphatase which reflects osteoblastic activity, and a rise in urinary hydroxyproline in patients with Paget's disease who were immobilised. He also drew attention to the very high excretion of urinary hydroxyproline output in patients

with thyrotoxicosis even when there was no significant rise in the alkaline phosphatase. Klein and Curtiss (1964) took the view that urinary hydroxyproline was largely derived during the process of bone formation. However, they based their conclusions on the results obtained from injection of parathyroid hormone into rats, and these results were quite contrary to the results obtained by Henneman (1964) when he injected parathyroid into normal and hypoparathyroid subjects. The conclusions of Klein and Curtiss (1964) also depended upon the results obtained by treating patients suffering from rickets with vitamin D in whom they claimed there was a fall in both the alkaline phosphatase and urinary hydroxyproline. These results do not agree with our findings (Smith and Nordin, 1964; Nordin and Smith, 1965) in patients with osteomalacia who were treated with vitamin D and in whom there was a rise in the urinary hydroxyproline.

In the present study, there was a fall in the bone destruction rate measured with ^{45}Ca with a corresponding decrease in the urinary hydroxyproline ($P < 0.01$) (Fig 80).

The decrease in urinary hydroxyproline correlated inversely with increasing positive balance ($P < 0.01$) (Fig 79). During this study there was no change in the mineral transfer rate. The fall in urinary hydroxyproline was further confirmed in a group of patients on their home intake, on and off calcium supplements (Fig 84).

These findings suggest that the major component of the urinary hydroxyproline in osteoporotic patients comes from the destruction of bone. This does not of course exclude the possibility that some contribution is made to the urinary hydroxyproline from bone formation. It is also feasible that this contribution may be different in patients with abnormal conditions, such as Paget's disease.

In 106 subjects, 24 hour urine samples were collected while they were on their home intake. In these patients there was a tendency for the hydroxyproline to rise with age but this did not achieve significance. However, the urinary creatinine output was also measured and when the urinary hydroxyproline/creatinine ratio is plotted against age there was a significant increase in output with age ($P < 0.01$), the rate of change being 0.51% per year. If a correction were

to be applied for the loss of bone mass with age (about 1% per year) then the rate of fall would be higher. These results are compatible with the loss of bone being due to an increase in the rate of bone destruction per unit mass while the bone formation remained constant. This is in agreement with the results obtained by Jowsey (1966).

CHAPTER VII

DIETARY INTAKE, CALCIUM ABSORPTION AND URINARY CALCIUM EXCRETION IN RELATION TO BONE MASS AND AGE IN MEN AND WOMEN

It has been shown (Light and Frey, 1941; Greaves, Scott and Scott, 1959; Harrison and Fraser, 1960) that a variety of laboratory animals fed on a low calcium diet, but with adequate vitamin D and phosphorus, developed osteoporosis. Jowsey and Gershon-Cohen (1964) confirmed these findings in cats. They also demonstrated that cats made osteoporotic with a low calcium diet, and then given supplementary calcium therapy showed an increase in bone density. That a positive balance can be produced in osteoporotic patients by feeding supplementary calcium has been reported by a number of authors (Owen, Irving and Lyall, 1940; Anderson, 1950; Whedon, 1959; Harrison and Fraser, 1961; Nordin, 1962a). The relation between dietary intake and osteoporosis in man is therefore of considerable interest. Smith and Frame (1965) investigated 2,000 ambulatory women. They found a progressive loss in the vertebral density and cortical bone of the metacarpal and femur with age. They were not, however, able to demonstrate any relation between osteoporosis and calcium intake, the calcium intake being calculated from a diet history.

This study is in three parts. The first part is concerned with the investigation of the dietary intake of calcium, phosphorus, protein and vitamin D in normal female subjects, and in female patients referred to a special bone clinic because they were thought to have osteoporosis. On these same subjects, calcium absorption was estimated using an isotope of calcium, and the urinary calcium excretion was measured.

The second part of the study is an investigation of the relation between the dietary intake of calcium, phosphorus, protein and vitamin D and the X-ray indices of bone mass in the same normal subjects and also in the patients referred because they were considered to be osteoporotic.

The third part of the study is a similar investigation in normal male subjects, and a comparison between the males and females.

Subjects studied

Three groups of subjects were studied. The first was the control group of 152 normal female volunteers recruited from the relatives and friends visiting patients in the wards of the Western Infirmary, Glasgow (Group I - see Chapter I). Diet histories were available in 141 of these subjects. The second was a group of 97 patients referred for investigation and treatment of severe back pain or recent fracture, and who were thought to be suffering from osteoporosis by the referring physician or surgeon on examination of standard X-rays. These 97 patients represent the first 97 patients of the 136 osteoporotic subjects described in Chapter II. The third was the control group of 75 normal male volunteers, as described in Chapter I. Diet histories were available in 68 subjects.

Procedure

The subjects were interviewed and the standard questionnaire illustrated in Appendix III completed for the three groups of patients. Skeletal X-rays were taken and the Metacarpal Index, Femoral Index and Spinal Index, and Relative Vertebral Density measured as described in Appendix II (**Nordin, Barnett, Smith & Anderson**, 1966). A radiocalcium test of calcium absorption was performed

(Bhandarkar et al, 1961) (Chapter IV). Random urine specimens were taken on two separate occasions and the mean of the two calcium:creatinine ratios recorded (Nordin and Smith, 1965). Patients with secondary osteoporosis (Nordin, 1961) were excluded from the survey.

Diet histories, with particular reference to calcium, phosphorus, protein and vitamin D, were obtained from all the subjects by a dietitian especially employed for the purpose. The validity of the diet histories in terms of the weekly-weighed diet is being reported elsewhere (Nordin and Harrison). All the data were recorded and processed by a medical data processing system utilising magnetic tape record files and flexible search on routine statistical processing.

Results

a) Social class

The social classes of the normal female volunteers (Group I, Chap. I) and patients were recorded according to their husbands' occupations (or their own if widowed or single). The two groups are compared in Table XXXIII. The social classes of both the control group and the patient group are very similar. Both show a preponderance of subjects in social class 3 (skilled workers).

b) The menopause

The average age of the onset of the menopause in normals was 47 years and in the patient group, 46.6 years, the subjects who had had an artificial menopause being excluded. The difference was not statistically significant when tested by Student's 't' test. A higher proportion of the subjects in the patient group had had an artificial menopause (23%) as compared with the control group (10%). The average age at the time of the artificially induced menopause was 41 years in both groups. Eighty-one of the normal group were post-menopausal and 84 of the patient group.

c) Incidence of fractures

This is shown in Table XXXIV. The incidence of fractures is significantly higher in the patient group than in the controls. In both groups, the highest incidence of fractures was in the upper limb.

d) Incidence and site of backache

The incidence and site of backache in the two groups is shown in Table XXXV. Backache affected 34% of the control patients and 92% of the referred patients. The patient group were older and, as can be seen from Table XXXVI, the incidence **is highest from 41-50 years.** No relation between backache and the X-ray score was found. The pattern in the two groups is very similar, the commonest site of back pain being the lumbar region, followed by a high incidence of pain in that site radiating down the legs, suggesting the presence of a disc lesion. Pain in the sacral region was surprisingly common. Pain in the thoracic region and cervical region were less frequently complained of.

e) Other clinical features

These are shown in Table XXXVII. The high incidence of urinary symptoms was striking. Four patients from each group complained of recurrent diarrhoea. Two subjects in each group had had renal stones, and three subjects in the patient group and one in the control group complained of loin pain at the same time in the past, suggestive of renal colic. The incidence of symptoms was slightly higher in the patient group than in the controls, but again the pattern was very similar.

f) Obstetric history and breast-feeding

In patients over 50 years of age, who would be expected to have reached the end of their reproductive life, the average parity in the normal group was 2.0 and in the osteoporotic group, 2.2. Of the subjects under 50 years of age, the mean parity in both the controls and the patient group was 2.2. In Table XXXVIII the mean parity in each of the decades between the 3rd and 7th decades, and in subjects over the age of 70 years is shown. Again, there was no significant difference between the groups. The average number of months that each subject with one or more children breast-fed their offspring in the normal group was 16.7 (S.E. = 1.35) and 20.4 (S.E. = 2.95) in the patient group. There was no significant difference between the two groups ($P > 0.05$).

g) Comparison of X-ray indices between normal subjects and patients, and the relation of the X-ray indices to age

The X-ray indices were correlated with age (Table XXXIX). It is clear, except for the spine index in the osteoporotic group, that X-ray indices of bone mass fall significantly with age in both groups. The two groups were compared by relating the Metacarpal Index, the Femoral Index, the Spine Index and the Relative Vertebral Density to age in 5 year age groups. The results of the comparison relating the Metacarpal Index to age are shown in Fig 85. As can be seen, there was no significant difference at any 5 year age group between the two groups though the patient group were slightly lower than the control group at each age. Similarly, when the two groups were compared in terms of the Femoral Index, Spine Index and Relative Vertebral Density, no significant difference at any age range emerged.

h) The relation between age, weight and dietary intake

The mean and two standard error range of intake of calcium (mg/day), phosphorus (mg/day), protein (gm/day), and vitamin D (International Units/day) in the normal and osteoporotic groups are shown in Fig 86. There was a significantly lower intake of phosphorus ($P < 0.001$), protein ($P < 0.001$), and vitamin D ($P < 0.001$) in the patient group, but the intake of calcium was not significantly different in the two groups. As body weight might be expected to influence dietary intakes, the weights of the two groups were compared. The mean weight of the normal women was 62.0 Kg ($S.E. = 0.93$), and of the osteoporotic women, 58.0 Kg ($S.E. = 1.10$). The 'osteoporotic' patients were therefore slightly but significantly lighter in weight than the normal subjects (Fig 87).

The age distribution of the normal and osteoporotic subjects were compared. The patient group were found to be significantly older than the control group, the average age of the osteoporotic patient being 61.8 years, and of the control subjects, 51.6 years (Fig 88). The body weights were regressed against age in both groups separately and

together. There was a slight but not significant fall in body weight with age (normal subjects r = -0.035, osteoporotic subjects r = -0.058 - normal subjects plus osteoporotic subjects r = -0.107). Since the patient group is both older and lighter than the control group, the dietary intakes were adjusted to take this into account and the results are shown in Fig 89 . As can be seen, there is no significant difference between the groups when studied in this way.

In both groups, the dietary intakes appeared to fall with age and this was further investigated. The results are shown in Table XL. The dietary intakes are correlated with age in both groups separately and combined. The analysis was repeated after the dietary intakes were divided by body weight. The intakes of phosphorus, protein and vitamin D, but not the intake of calcium, fell significantly with age in the control subjects. However, when the dietary intake was divided by body weight no significant relation between intake and age was found. In the patient group, only the protein intake, not divided by body weight, showed any significant fall with age. When the groups were combined, all dietary intakes

showed a small fall with age. When the intakes were divided by body weight, there was a significant fall in the intake of phosphorus, protein and vitamin D but not in calcium with age.

i) Calcium absorption

The absorption of calcium as measured by the radiocalcium absorption test is plotted against age in 10 year age groups, and the results are shown in Fig 90. There is no significant difference between the groups, except between 61 and 70 years, when the patient group showed a significantly lower calcium absorption. However, this difference was no longer apparent after the plasma specific activity was corrected for body weight. There was no significant fall with age when the groups were studied separately, but when both were taken together there was a small but significant fall in absorption with age ($r = 0.18$; $P < 0.01$). Since body weight would be expected to influence the measurement of the plasma level at 2 hours after ingestion of the radioisotope because the calcium pool would be larger in the heavier subject, the two hour serum value was multiplied by body weight. No significant difference emerged between the groups, but there was a significant fall in absorption when both groups were taken together ($r = 0.235$; $t = 3.512$; $P < 0.001$).

j) Urinary calcium excretion

The urinary excretion of calcium, as measured by the mean of two separate estimations of the calcium:creatinine ratio in the two groups, did not differ significantly (Fig 91). Again, when regressed against age, there was no fall in urinary calcium output with age when the groups were considered separately or together.

The relation between X-ray indices and nutrition in female subjects

It is evident that the normal subjects (Group I) and the patients referred because they were considered to be osteoporotic (Group II), do not differ significantly in their X-ray scores at any decade. The mean X-ray score of the patients in each decade is very slightly lower than that of the control group though this is not surprising in view of the way in which they were selected. Further, the rate of fall of bone mass with age in both groups is virtually identical. The age of onset of naturally occurring menopause was 46.6 years in the patient group and 47 years in the control group. Parity was similar in both groups. The length of time of breast-feeding was slightly but not significantly longer in the patient group than in the controls. The distribution of the social classes within the groups was very similar. The incidence of fracture and backache was higher in the patient group, but again this is not surprising as it was for these reasons in the main that the patients had been referred for investigation.

The dietary intakes in the two groups when adjusted for age and weight again do not differ significantly. Calcium absorption was very similar in the two groups except between 61 and 70 years when a small but significant difference was found. The importance of this, however, is doubtful as the mean absorption was virtually identical in all other decades. Furthermore, no significant difference in absorption at any decade was found when the plasma activity was corrected for body weight. The urinary excretion of calcium is again very similar in the two groups.

Since it is the fall in bone mass which is the important factor in determining whether or not fractures will occur, and both groups have similar bone mass on X-ray and in whom the bone mass falls at the same rate, studies of the effect of diet on the bone mass in the two groups have been combined and the bone mass and dietary intake compared.

Results

1. X-ray score and dietary intake

In Figs 92 to 95 the dietary intake of calcium, phosphorus, protein and vitamin D have been plotted against the X-ray indices. The dietary intakes of calcium and phosphorus are plotted in 200 mg intervals, vitamin D in 10 International Unit divisions and protein in 10 gm intervals. Since it is evident that body weight is an important variable, the intake of each of the constituents has been divided by body weight and the results are shown in Figs 96 to 99.

The correlations between dietary intake and X-ray scores are shown in Tables XLI, XLII, and XLIII.

Tables XLI and XLII show the relation between the various X-ray indices in the normal and 'osteoporotic' female subjects separately. Before dividing the dietary intake by body weight there is a positive correlation between Metacarpal Index and calcium intake in both groups. The Metacarpal Index in both groups and Relative Vertebral Density in the normal group are related to phosphorus intake. There is no relation between the X-ray indices and protein or vitamin D intake in the 'osteoporotic' subjects, but in the normal group all the indices except the Spinal Index are related to protein intake. The Spinal Index and Relative Vertebral Density are related to vitamin D intake in the normal subjects. When the dietary intakes are divided by body weight there is no relation between the X-ray indices and intake in the patient group. In the normal female group there is a relation between Metacarpal Index and phosphorus and protein intake, and Relative Vertebral Density and vitamin D intake only. When the two groups are combined there is a significant relation between all the X-ray indices and protein intake; between all the indices except the Femoral Index and vitamin D intake, and between all the indices except the Spinal Index and phosphorus intake. Only the Metacarpal

Index is weakly related to calcium intake. When the dietary constituents are divided by body weight there is no relation between calcium intake and the X-ray indices. There is weak relation between Metacarpal Index and phosphorus intake. The protein intake is related to the Metacarpal Index and Relative Vertebral Density. Vitamin D intake is related to all the X-ray indices except the Femoral Index.

The X-ray indices fall with age (Table XXXIX). There is also a fall in dietary intake with age (Table XL). There is a slight though not significant fall in body weight with age. When the dietary intake is divided by body weight then there is still a significant fall in dietary intake with age of phosphorus, protein, and vitamin D (Table XL).

Therefore, age is the major factor determining the value of the X-ray indices, and the data is re-analysed in Figs 100 to 111. These figures illustrate the dietary intake of calcium, phosphorus, protein and vitamin D in the combined group, the subjects being divided into decades from the 5th to the 7th. In each decade, the subjects were divided into those having high and low X-ray scores the mean being the dividing line. The dietary intake of these subjects with

high and low X-ray indices have been compared. This comparison was made before and after dividing the dietary intake by the body weight.

Between the ages of 41 and 50 years, there was no relation between the dietary intake and the Metacarpal Index (Fig 100). There was a significantly lower intake of phosphorus and protein in those patients with low femoral indices which was no longer apparent when the individual dietary intakes were divided by body weight (Fig 101). There was a significantly lower intake of phosphorus/Kg body weight in those patients with spinal biconcavity (Fig 102). There was however no difference between the dietary intakes of those patients with low and high Relative Vertebral Densities, (Fig 103).

In the 6th decade, those subjects who had a low Metacarpal Index had a significantly lower intake of calcium, phosphorus and protein than those with a high Metacarpal Index (Fig 104). Only the difference in protein intake remained significant when the dietary intake was divided by body weight.

There was no significant difference in intake of any dietary constituent in those subjects with high or low femoral indices (Fig 105). There was no evidence of a lower dietary intake in those subjects who had spinal biconcavity (Fig 106). However, those subjects who had a low Relative Vertebral Density had a significantly lower intake of phosphorus, protein and vitamin D whether the dietary intake was divided by body weight or not (Fig 107).

In the age range 61 to 70 years, it was only in those subjects who had spinal biconcavity (i.e. a low Spinal Index) that there was a slightly lower intake of vitamin D (Fig 110), but this difference was no longer apparent when the vitamin D intake was divided by body weight (Fig 110). There was no significant difference between the dietary intakes and the subjects with high or low metacarpal, femoral or spinal indices or Relative Vertebral Densities, (Figs 108, 109, 111).

A comparison between the dietary intake, calcium absorption, urinary calcium excretion and bone mass in male and female subjects

It is evident that the major problem of osteoporosis in the ageing population is the bone loss that affects women. There is a much smaller loss of bone in men with age as has been shown in Chapter I and as has been illustrated in studies by Gitman and Kamholtz (1965), Morgan et al (1967), Garn, Rohman and Nolan (1964), and Meema, Bunker and Meema (1965). Moreover, the importance of this difference is evidenced by the higher incidence of fractures in women and the much higher rate of increase in the prevalence of fracture with increasing age (Chapter III). This increase appears to be directly related to the rate of bone mineral loss up to the age of 75 to 80 years after which the rapid increase in fracture incidence is obviously related to factors other than the rate of bone mineral loss which tends to decrease after the age of 70 years.

It is evident therefore that a study of the differences between the dietary intake, calcium absorption and urinary excretion of calcium in male and female subjects could be of considerable interest. This study is concerned with the investigation of dietary intake, calcium absorption, urinary calcium excretion and bone mass in male and female subjects.

Subjects studied

The female subjects are the combined normal group and the subjects referred to a special Bone clinic because they were considered to be suffering from osteoporosis. Both groups of subjects have been examined in the present chapter and were not found to differ significantly. The reasons for regarding them as a normal population has been discussed.

The male subjects were those of Group II (Chapter I) and consisted of 75 subjects, as described on p. 37.

The procedure followed for the investigation of these two groups has been described on p. 191.

Results

The male subjects are significantly younger (Fig 112) and heavier (Fig 113) than the female subjects.

In Chapter I, the social classes of the groups have been examined and there is a higher percentage of subjects in Social Class 3 in the female subjects.

The changes with age in each group of the Metacarpal Index (M.I.) the Femoral Index (F.I.), Spinal Index (S.I.) and Relative Vertebral Density (R.V.D.) are shown in each group (Table XLIV). Only the fall in the Femoral Index in the male subjects reaches significance ($P < 0.01$). All the X-ray indices fall significantly with age in the female subjects ($P < 0.001$ in each instance).

Since there are significant differences in age between the male and female subjects (Fig 112) they have been divided into groups by decades and the X-ray indices then compared. The results are shown in Figs 114 to 117. There is a relatively small fall in the X-ray indices with age in the male subjects, but even so significant differences between the males and females were only apparent after the age of 60 years.

Further, this was only evident in the M.I. and R.V.D., though the pattern of change was similar in the S.I. and F.I..

The male subjects had a significantly higher intake of calcium, phosphorus, protein and vitamin D than the female subjects ($P < 0.001$ in each instance), but this difference was no longer apparent when the dietary intakes were divided by body weight (Figs 118 and 119). Since the male subjects were younger on average than the female subjects, the dietary intake was examined by decades (Figs 120 to 123). There was a significantly lower intake of protein at all ages in the female subjects and in vitamin D in the 5th and 6th decades. However, these differences were no longer apparent when the dietary intakes were divided by body weight (Figs 124 to 127).

Calcium absorption falls significantly with age in the female subjects when considered as the 2 hour plasma specific activity and as the plasma specific activity multiplied by body weight to correct for differences in size of individuals. The calcium absorption shows no significant change with age in the male subjects either considered as the 2 hour plasma specific activity or as this value corrected for body weight.

The calcium absorption in the male and female subjects has been examined in each 10 year decade and the results are shown in Fig 128 and 129. When considered as the specific activity at 2 hours (Fig 127), the female subjects between 51 and 60 years and 61 and 70 years have a significantly lower absorption of calcium than the males. When the value is corrected for differences in weight of individuals, the female subjects have a significantly lower absorption throughout the age range 31 to 70 years (Fig 129).

The urinary calcium excretion measured as the calcium/creatinine ratio shows no significant fall with age. When compared with the female subjects in 10 year decades again there is no significant difference between the male and female subjects, except between 31 - 50 years. However, the male subjects have a lower mean Ca/Cr ratio throughout the age range from 31 to 70 years (Fig 130).

The relation between dietary intake of calcium, phosphorus, protein and vitamin D, and the X-ray indices of bone mass in the male subjects are examined in Table XLV. In contrast to the female subjects, it is only the Femoral Index that shows a significant correlation with dietary intake of calcium, phosphorus and protein, but not of vitamin D. When the intakes of the various constituents are divided by body weight there is a weak positive correlation between the intake of calcium and phosphorus with the Relative Vertebral Density.

There is a small fall in the dietary intakes of calcium, phosphorus and protein with age in the male subjects (Table XLVI) but it is only the fall in protein intake that achieves significance ($P < 0.05$). There was a slight fall in body weight with age ($r = -0.225$) but this did not reach significance ($T = -1.949$; $P > 0.05$).

Discussion

The striking feature in this study is the similarity between the two groups of female subjects selected in completely different ways. The control subjects were selected as described previously (p. 31) by approaching the relatives and friends visiting patients in the general medical wards of the Western Infirmary, Glasgow. The patient group consisted of patients who had been referred to a special Bone clinic because they were thought to be osteoporotic. All had presented with symptoms such as backache or had had a recent fracture, and the referring physician or surgeon had felt that the patient had osteoporosis after the X-rays had been examined. All the patients had serum calcium and phosphorus estimations, and a calcium:creatinine ratio and phosphate excretion were estimated by measuring the Phosphate Excretion Index (P.E.I.) (Nordin and Fraser, 1960). In this way, patients who had evidence of other bone disease were excluded.

The distribution within the social classes of the two groups was very similar, both showing a higher proportion of patients in social class 3 (skilled workers) which reflected the population served by the hospital at the time of the investigation.

The average age at the onset of the menopause was not significantly different. A higher proportion of the subjects in the patient group had had an artificial menopause, but the operation is done more frequently in patients who are approaching or have passed their menopause. The patients constituted an older group so that this finding is not surprising. The patient group had a higher incidence of fracture and of backache. Again this is not a surprising finding. There were two reasons for this, the first being that these two features were the commonest reasons for referring the patients, and the second that the patient group were older. When symptoms were considered there was a slightly higher incidence of other symptoms in the patient group, but the differences were small. The striking feature is the high incidence of urinary symptoms in both groups. Parity did not differ significantly in either group considered as the mean or by decades. There was no difference in the average length of time that either of the groups had breast-fed their infants.

Though the patient group had significantly lower X-ray indices and densities than the control group, they were older, and it is now clearly established that X-ray indices fall with age (Meema and Meema, 1963; Nordin et al, 1966; Fujita, Orimo and Yoshikawa, 1966; Morgan et al, 1967; Smith et al, 1969). Once age was allowed for, there was a striking similarity between the groups in the measurements of bone mass. Both groups also showed a fall in bone mass with age.

The dietary intakes of the two groups showed a significantly lower intake of phosphorus, protein and vitamin D, but not of calcium in the patient group. However, the patient group constituted an older and lighter population and once age and body weight were allowed for, these differences disappeared. The two groups did not differ in their absorption of calcium as measured by an isotope test of absorption using ^{45}Ca, and no difference was found in the urinary output of calcium as measured by the mean of calcium:creatinine ratios in the urine.

There was a slight fall in calcium intake with age. There was a more significant fall in the intake of phosphorus, protein and vitamin D with age. There was a very significant fall in bone mass with age. Though these dietary changes with age are small, only a small negative balance of less than 30 mg/day of

calcium would be enough to account for the loss of bone in these subjects. However, this does not necessarily mean that dietary intake is related to the development of osteoporosis, as both the fall in X-ray measurement and dietary intake could be a function of ageing.

In this investigation, which is concerned with the relation between diet and bone mass, the patients and the normal female subjects were considered together. The reasons for considering them as forming a single population have been discussed and it is evident that once age and body weight are allowed for, that they do not differ significantly. The dietary intake of protein, phosphorus and vitamin D are significantly related to the X-ray indices of bone mass. Calcium intake is only weakly related to the X-ray indices. There was a more significant relation between the X-ray indices and the intake of phosphorus, protein and vitamin D. However, these differences could result simply from the fall in the dietary intake with age and be co-incidental to the fall of the X-ray indices with age. That is, the fall in both could be simply due to ageing or some unknown factor associated with ageing.

The only way to eliminate this effect so that the importance of diet can be considered is to examine the dietary intake in subjects with high and low X-ray indices and Relative Vertebral Densities. The results are illustrated in Figs 100 to 111. Only minor differences, which decreased markedly when body weight was allowed for, were evident in the 5th and 7th decades. In the 6th decade, the differences in intake were more significant. There were small differences in calcium and vitamin D intake, but more significant changes in the intake of phosphorus and especially of protein. There is no immediately obvious explanation of why this should be so, but it is interesting that it is at this age that the rate of loss of bone is greatest in women. The menopause occurs at 47 years of age on average, and the X-ray indices fall markedly in the period 5-10 years after the menopause.

It is not an artefact due to there being a higher proportion of larger individuals in one or other group as these differences persist though the significance is decreased by dividing the dietary intake by body weight. Further, the X-ray measurements of bone themselves allow for differences in the size of individuals (Chapters I, II and III).

However, it would be only too easy to over-emphasise these differences, and the mean dietary intakes would be considered completely adequate by the recommended intake of nutrients for the United Kingdom laid down by the Department of Health and Social Security (H.M. Stationery Office, Report on Public Health and Medical Subjects No. 120). This study emphasises the importance of allowing for the changes in body weight and bone mass with age. It is evident that the dominant factor concerned with the loss of bone in women is age or some factor associated with age.

In Chapter III, the importance in the difference in the changes in bone and its association with fracture in males and females has been discussed. The incidence of fracture increases in men and women with age, but the rate of this increase is very much higher in women. This is directly related to the total cortical mineral content, which falls much faster in women than in men. The differences between the mineral content in male and female subjects, once age and size have been allowed for, become significant after the 6th decade. The male subjects have a significantly higher intake

of calcium, phosphorus, protein and vitamin D. However, once allowance has been made for differences in weight, and the intakes of the various dietary constituents considered by decades, there is no significant difference between male and female subjects. This adds further support to the evidence that the development of primary osteoporosis is not related to dietary intake of calcium, phosphorus, protein or vitamin D in this Western urban population.

There is, however, a small but significant fall in calcium absorption with age in women, which does not occur in the male subjects. There is a small, but again significant fall in dietary intake, though there is no appreciable difference between the male and female subjects. There is no significant fall in urinary calcium excretion with age in the female subjects in spite of the diminished calcium intake and calcium absorption. The male subjects do not show a significant change in urinary calcium with age, but they do show a slightly lower calcium excretion than the female subjects at all ages, though this does not reach significance, except **in the 4th and 5th decades.**

Conclusions

The fall in X-ray indices of bone mass with age in women is associated with a fall in the dietary intake of calcium, phosphorus, protein and vitamin D. The smallest decrease in intake with age was in the calcium intake. However, the female subjects show a fall in calcium absorption with age, while the urinary calcium excretion remained unchanged up to the age of 70 years. These changes were not observed in men who show relatively small changes in bone mass with age. The intake of calcium, phosphorus, protein and vitamin D were very similar in male and female subjects once age and differences in body weight were allowed for. Moreover, the male subjects had a significantly better calcium absorption than the female subjects at each decade between the ages of 30 and 70 years. The male subjects also had a lower urinary calcium excretion at each decade between the ages of 30 and 70 years, though these differences did not achieve significance at any one decade. These differences between the male and female subjects would be sufficient to account for the differences in the X-ray indices which develop with age. There are also significantly smaller intakes of phosphorus, protein and vitamin D in the female

subjects between the ages of 51 and 60 years, who have lower spinal densities. It is interesting that these differences in the spine should occur in trabecular bone in the main at the decade following the menopause. However, none of the above associations need imply causation. Most of the results, except the last, could be explained as being secondary to changes at the cellular level in bone, or in the factors, such as parathyroid secretion or the activity of calcitonin and growth hormones, which control bone formation and destruction.

CHAPTER VIII

BONE DENSITY AND CALCIUM METABOLISM IN PATIENTS FOLLOWING VAGOTOMY WITH GASTRO-JEJUNOSTOMY AND PARTIAL GASTRECTOMY

In 1939, Ask-Upmark predicted that partial gastrectomy might give rise to osteomalacia. Nicolaysen and Regaard (1955) found a negative calcium balance in some gastrectomised patients. Osteomalacia, resulting from gastrectomy, has now been reported by a number of authors (Baird and Oleesky, 1957; Clark, Crooks, Dawson and Mitchell, 1964; Hall and Neale, 1963; Melick and Benson, 1959; Harvald, Krogsgaard and Lous, 1962). Steatorrhoea is known to occur in a proportion of patients following gastric surgery, and distrubances of calcium metabolism are inferred to arise from this source. However, a decrease in appetite is common following gastrectomy, and weight loss is usual (Avery Jones, Gummer and Lennard-Jones, 1968). Ekbom and Hed (1965) report on a series of 35 partial gastrectomised patients selected from a series of 317 patients because they had a history of malabsorption, i.e. anaemia, weight loss and recurrent diarrhoea. Of these, 15 consecutive patients were studied in detail. Seven had increased calcium retention following calcium infusion. Two patients had raised alkaline phosphatase. Eight patients had definite steatorrhoea with faecal fats of more than 9 gm/day and a further three patients had faecal fats

greater than 5 gm/day. X-rays showed thinning of the bones in only three patients and this was mainly of the vertebral column. None had spontaneous fractures or Milkman's fractures. All the patients were unable to tolerate milk and some could not tolerate cheese either. Calcium intake in gastrectomised patients 199 - 1066, mean 649 $\pm$ 6 <. Controls 604-1135, mean 883 $\pm$ 41. The difference between the two groups was highly significant ($P < 0.001$). Deller and Begley (1963) found 'decalcification' in 50% of their patients - 100 patients, and 100 controls. Clark et al (1964) found metabolic calcium disturbance in 28% of 55 patients. Thompson, Lewis and Booth (1966a) reported a diminished absorption of vitamin D using trittium labelled vitamin D in 5 out of 6 patients with osteomalacia following gastrectomy. The absorption was virtually nil in one patient with associated pancreatic defect. The net absorption of vitamin D was normal in 4 patients following gastrectomy without osteomalacia, two of whom had frank steatorrhoea. Thompson, Neale, Watts and Booth (1966b) reported further investigations in 28 out of 200 patients who had a raised alkaline phosphatase following gastric surgery. Three had hepatic

dysfunction, six had Paget's disease and ten had osteomalacia. Most of the remainder had abnormal calcium infusion tests. They concluded that in the absence of hepatic dysfunction and Paget's disease, a raised serum alkaline phosphatase is usually indicative of either overt or sub-clinical osteomalacia.

Deller and Begley (1963) and Clark et al (1964) reported abnormally high values of alkaline phosphatase in about 15% of patients who had undergone gastric surgery. However, Morgan, Paterson, Woods, Pulvertaft and Fourman (1965a) found a raised alkaline phosphatase less frequently than these workers and demonstrated that other causes were responsible for the rise in alkaline phosphatase in a number of patients. They found (1965b) osteomalacia in only 3% of women and less than 1% of men following gastric surgery. These authors suggested that the cause of osteomalacia was most probably due to vitamin D deficiency. Clark and Crooks (1965) are critical of the work of Morgan et al (1965a) on the grounds that they attempted to show response to vitamin D therapy in patients by a fall in alkaline phosphatase, but some of their patients had normal serum alkaline phosphatase before the investigation was started, and pointed out that early cases of osteomalacia might be missed if a low serum calcium was

considered to be invariably present in osteomalacia.

Another source of alkaline phosphatase in the serum is the intestine. Pulvertaft, Luffman, Robson, Harris and Langman (1967) investigated this further as it had been suggested by Bamford, Harris, Luffman, Robson and Cleghorn (1965) that the higher alkaline phosphatase in patients with gastrectomy might reflect the contribution from the intestinal isoenzyme component. However, they concluded that the intestinal component of the alkaline phosphatase in the serum of patients operated upon for peptic ulceration were affected to much the same degree by diet, and ABO blood grouping, and secretory status as in healthy individuals.

Surprisingly little interest has been paid to the dietary intake of calcium, phosphorus and vitamin D in patients who have undergone gastric surgery. Moreover, it is evident that the type of gastric surgery might well influence the outcome in terms of changes in skeletal metabolism. It is proposed therefore to consider the effects of vagotomy with gastro-jejunostomy and poly-partial gastrectomy and diet on calcium metabolism.

Patients studied

The study was started in the Western Infirmary, Glasgow, in 1954. Male patients attending for elective surgery for duodenal ulcer were allocated randomly for treatment to either operation. Only patients who had been operated on at least eight years previously were included in the investigation, and the details of the patients so chosen are shown in Table XLVII.

Fifty-six of the patients had undergone a polya-type gastrectomy. Fifty-one had a vagotomy with gastro-jejunostomy performed. These represented 82% and 84% of the patients available for the study. The mean age, height and weight were very similar in each group. The mean time elapsed since operation was again strikingly similar (Table XLVII).

The control group of subjects consisted of the 75 normal male subjects identified as Group II in Chapter I and discussed in detail in Chapter VII.

Methods

X-rays

Skeletal X-rays were carried out in order to determine the Metacarpal Index, Femoral Index and Spinal Index (Barnett and Nordin, 1960). The serum alkaline phosphatase was determined by the method of King and Wootton (1956). The serum and urinary calcium were determined by titration with EDTA using ammonium purpurate as an indicator (Nordin and Smith, 1965). The determination of serum and urinary phosphorus and creatinine were carried out by standard AutoAnalyzer techniques (Technicon Series N4a and N11a). Dietary histories and weekly-weighed diets were carried out by a dietitian especially employed for this purpose.

Results

The serum calcium was very slightly reduced in one out of 51 patients who had undergone vagotomy and gastro-jejunostomy. It was also slightly reduced in 5 of the patients who had had a polya-type gastrectomy (Fig 131), the lower limit of normal by the method employed being 8.9 mg% (Nordin and Smith, 1965).

The serum calcium was not related to age or to the time since operation in either group taken separately or when both groups were taken together, nor was it related to the dietary intake of either calcium or phosphorus.

The serum phosphorus is shown for both groups in Fig 131. It was normal in all of the 53 patients in whom it was measured who had undergone polya-gastrectomy. It was slightly reduced (2.4 mg%) in one patient who had had a vagotomy and gastro-jejunostomy. There was no significant difference in the serum phosphorus between the two groups. There was no relation between the serum phosphorus and the dietary intake of phosphorus either separately or when taken together. There was also no relation between the plasma phosphorus and the time since operation.

The alkaline phosphatase was raised in 5 of the 53 patients who had had the polya-type gastrectomy, and it was raised in 5 of the 51 patients who had undergone vagotomy and gastro-jejunostomy (Fig 131). The alkaline phosphatase was not significantly different in the two groups. It was not related to dietary intake of calcium or phosphorus, or time since operation i

either group taken either separately or both groups taken together. It was not related to age when taken separately, but there was a weak positive correlation with age when the data from both groups were combined ($P < 0.05$).

The urinary calcium:creatinine ratio was slightly raised in 2 of the patients who had had a vagotomy and gastro-jejunostomy, and 3 patients who had undergone a polya-gastrectomy. In none of the patients was the urine calcium:creatinine ratio below the lower normal level of 0.03 (Nordin and Smith, 1965) (Fig 132). The urinary calcium output was not related to dietary intake in either group and was not related to the time since operation or to age.

The urinary phosphate excretion, measured as the Phosphate Excretion Index (P.E.I.) was raised in 11 patients who had had a vagotomy with gastro-jejunostomy and in 15 subjects who had undergone a polya-gastrectomy. There was no direct relation between phosphate excretion and diet, age, or time since operation in either group considered separately or combined. There was no significant difference in the P.E.I. of the two groups (Fig 132).

The dietary intake of calcium and phosphorus is considered in relation to age measured as the intake of each in mg/day (Figs 133 and 134) and in mg divided by body weight (Figs 135 and 136). The calcium and phosphorus intake are shown in each 10 year decade from 31 to 70. In each decade, the detary intake of the normal subjects is shown first and is followed by the intake in the group who had had a vagotomy with gastro-jejunostomy followed by the intake in the patients who had had a polya-gastrectomy. The last column in each decad

shows the intake of the two patient groups taken together. The mean and two S.E. range is shown in each case. There was no significant difference between the intake of either calcium or phosphorus when the two patient groups were compared. However, the mean intake of calcium and phosphorus was higher in the patients, taken either separately or together than the intakes in the corresponding age groups in the normal subjects. In a number of instances where numbers and similar variances permitted comparison, the patients who had undergone gastric surgery had a significantly higher intake of both calcium and phosphorus than the normal subjects. The significance of the results are detailed in Table XLVIII.

Each patient who was found to have an abnormal biochemical result was sent an appointment to re-attend the clinic for further investigation. Five patients in each group had a raised alkaline phosphatase (Table XLVIX). Seven of the ten patients had only marginally raised values (13-14 King Armstrong Units/100 ml) and on re-testing on two or three separate occasions these were all found to be within the normal range (Table XLVIX). One patient with a

significantly raised alkaline phosphatase failed to re-attend. His other biochemical tests and bone X-ray indices were normal. A patient (R.M.) with vagotomy and gastro-jejunostomy had a persistently raised alkaline phosphatase, with no evidence of liver disease, which did not respond to oral vitamin D. The remaining biochemical tests were normal apart from a raised urinary hydroxyproline excretion; the presence of Paget's disease of bone was considered to be the most likely explanation for the raised alkaline phosphatase. A patient (W.S.) with a persistently raised alkaline phosphatase who had had a polya-gastrectomy had a persistently low serum calcium and raised phosphate excretion. Bone biopsy showed no diagnostic broadening of the osteoid borders, but following vitamin D therapy, all his biochemical values returned to normal, and the evidence in his case therefore strongly favours a diagnosis of osteomalacia. Yet another patient with a vagotomy and gastro-jejunostomy, and three patients with polya-gastrectomies who had low serum calciums failed to re-attend. Of the remaining three patients (W.S.) as described above almost certainly had osteomalacia. The other two patients had normal serum calciums on re-attending, (Table XLVIX). The diagnoses in the three remaining patients who

failed to return therefore remains uncertain, but their other biochemical tests were normal, and though osteomalacia could not be excluded, another explanation which remains possible is that their plasma proteins may have been low.

Eleven patients who had had a vagotomy with gastro-jejunostomy and fifteen patients who had had a polya-gastrectomy had raised phosphate excretions. The tests were carried out when on their home intake and the possible explanation lay in a high phosphate intake (Table L). All except three patients had a high phosphate intake. These patients were requested to re-attend the clinic, but four failed to do so. When the tests were repeated after an overnight fast all but three had a normal phosphate excretion. Of these three patients one (W.S.) was osteomalacic, one (L.B.) had evidence of early renal failure (blood urea 42 - 45) which is known to raise the P.E.I. (Nordin and Smith, 1965), and the remaining patient (C.N.) had a persistently low plasma phosphorus in addition to a raised P.E.I.. Both these values returned to normal after treatment with vitamin D. Of the four patients who failed

to re-attend, all had normal biochemical values though in one patient the serum calcium was at the lower limit of normal.

Two of the patients therefore had good evidence of osteomalacia as judged by their response to therapy. Eight patients who had one abnormal biochemical test - either a raised alkaline phosphatase, low serum calcium or raised phosphate excretion failed to re-attend for further investigation. Four of these patients had marginally raised phosphate excretions though their other tests were completely normal and the most likely explanation in this instance is the high dietary phosphate intake. Three patients with low serum calciums failed to re-attend. Their other biochemical tests were normal. One patient with a significantly raised serum alkaline phosphatase failed to re-attend; again his other biochemical values were completely normal.

X-ray indices

Since age is an important factor in the loss of bone this has been examined and the results are tabulated in Table LI. The Metacarpal Index fell significantly with age in the two patient groups, but not in the normal subjects (Table LI and Fig 137). There was a significant fall with age in the Femoral Index of the normal subjects, but not in either of the patient groups or in the combined patient group. Neither the normal subjects nor the patient groups showed any significant increase in spinal biconcavity with age as evidenced by the Spinal Index (Table LI).

Since age is the dominant factor determining bone mass in order to compare the patient group and the normal subjects in Group II, all subjects have been divided into 10 year age groups and the X-ray indices have been compared. The results are illustrated in Figs 138, 139 and 140 . The significance of the difference has been examined by the Student's 't' test and are shown in Table LII . There was no significant difference between the patient groups for any of the X-ray indices in any of the decades tested (Table LII). There

were small but significant differences between the normal subjects and the patients who had had a vagotomy and gastrojejunostomy, and the patients who had had a polyagastrectomy (Table LII). When the two patient groups were combined there was a significantly lower Metacarpal Index between 51 and 70 years, and in the Femoral Index between 50 and 60 years.

Discussion

The estimations of serum calcium and phosphorus, and urinary calcium and phosphate excretion were carried out with two objects in view. First, was to make a diagnosis of osteomalacia if this was present. Second, was to determine whether the serum values of calcium and phosphate excretion were related to dietary intake. If the second objective was to be achieved, it appeared more reasonable to do the serum and urine estimations without fasting the patient, but to collect all the specimens between 11 a.m. and 12.30 p.m. between which times the patients attended a special clinic. Since diet can alter the biochemical values, any of the patients who had values outside the normal range were re-tested after an overnight fast.

A low serum calcium was found in one patient who had undergone a vagotomy with gastro-jejunostomy and in five patients who had undergone a polya type gastrectomy. There was a slightly reduced serum phosphorus in one patient who had undergone a vagotomy with gastro-jejunostomy. There was no relation between the dietary intake of either calcium or phosphorus and the serum values and the time since operation, but this result should be interpreted with caution as variations in dietary intake might have disguised any trends present: The groups were considered

both separately and together when the above relations were looked for.

The serum alkaline phosphatase was raised in five patients in each group. It was not related to dietary intake or to the time since operation when the two patient groups were considered both separately and together. There was a weak positive correlation with age when the data from both groups were combined.

The urinary calcium excretion was not related to diet, age or time since operation in the groups considered separately and together. Six of the patients had a raised urinary calcium (calcium/creatinine ratio greater than 0.28) but this was not related to any other specific biochemical abnormality. No patient had a calcium/creatinine ratio below 0.03 which would be considered abnormally low (Nordin and Smith, 1965). Phosphate excretion as measured by the P.E.I., (Nordin and Fraser, 1960) was raised in eleven patients who had undergone a gastro-jejunostomy with vagotomy and in fifteen of the patients who had undergone a polya-type gastrectomy. Though the P.E.I. did not correlate with diet, it would seem likely that the high phosphate intake in these patients could well have been responsible for these raised values.

In the final analysis, one patient from each group had good evidence of osteomalacia; they had at least two persistently abnormal biochemical tests which returned to normal after treatment with vitamin D was started. One patient had early renal impairment (Blood urea between 42 and 45 mg%). One patient had a persistently raised alkaline phosphatase which failed to return to normal following treatment with vitamin D, and though there was no X-ray evidence of Paget's disease, this was presumed to be the most likely diagnosis since liver function tests were repeatedly normal.

All the other patients had normal biochemical values on repeat testing. However, eight patients failed to re-attend in spite of many attempts to get them to do so. Of these, four had raised phosphate excretions, but all their other biochemical values including the serum creatinine were within the normal range. Of the remaining subjects, one had marginally low serum calcium, though all his other biochemical tests, including the alkaline phosphatase, were normal. One patient with an elevated alkaline phosphatase failed to attend though again all his other biochemical data were normal.

These results indicate an incidence of osteomalacia in at least 2% of these patients. It seems unlikely that the remaining eight subjects had osteomalacia, but the possibility cannot be excluded and this would indicate a maximum value of 10%. However, minor degrees of osteomalacia might not be detected by these tests. Anderson, Campbell, Dunn and Runciman (1966) have shown that any or all of these values might be normal in patients in whom the criteria for osteomalacia might be satisfied on bone biopsy or the retention of calcium following a calcium infusion. One of the patients studied (W.S.) who had biochemical evidence of osteomalacia and whose biochemical tests returned to normal following vitamin D therapy did not have osteoid borders in the bone biopsy specimen examined. It is evident therefore that early or slight deficiency of vitamin D need not result in the production of criteria which would satisfy a diagnosis of osteomalacia and the warning of Clark and Crooks (1965) would appear to be a timely one.

It is further evident that in spite of an intake of calcium and phosphorus, greater on average than that in the normal population, that the X-ray indices were lower in the patient groups than in the normal subjects. The vitamin D intake was

not assessed in these subjects, but the main dietary constituent which raised the intake of calcium and phosphorus was milk, which would contribute significantly to the vitamin D intake. The finding of this high dietary intake is in direct contrast to the findings of Ekbom and Hed (1965). It was clear from questioning these patients that this high dietary intake had started following dietary advice before they came to operation, and they had continued to take a larger than average amount of milk after the operation.

Since the bone mass is lower in the patient groups than in the normal subjects, and if dietary constituents are the cause of the thinning of bones, then absorption of calcium and possibly of phosphorus might well be the factors responsible. Thompson et al (1966a and 1966b) found diminished vitamin D absorption in the patients who had undergone gastric surgery and had osteomalacia using tritium labelled vitamin D and even if the dietary intake was adequate, failure to absorb vitamin D could be a contributory factor. It is also evident that the older patient is more severely affected. There was no significant difference between the time since operation in the subjects above and below 50 years of age.

However, it is only after this age that the loss of bone

mass becomes an important factor. The time since operation would be expected to affect the bone mass, and the likely explanation for this not to be apparent is that all the subjects had been selected so that they had had their operations at least 8 years previously and none had had their operations more than 12 years prior to the investigation. The 4 years between may well be too small a range to allow for significant differences in bone mass to appear. However, as the time range since operation was sosmall between the two groups, it was possible to compare the effects of gastric surgery performed at different ages. It was evident that the older patients were affected most. The time since operation did not differ significantly between those patients under 50 years of age compared with those over 50 years. It was only after the age of 50 years that the differences between the patient groups and the normal subjects became evident. Before attaining the age of 50 years, the normal subjects and the patients with gastric surgery had very similar bone masses as measured by the X-ray indices described.

In conclusion, it would appear that gastric surgery results in the loss of bone to a greater extent than does occur in the normal population. Frank osteomalacia does occur but is relatively rare. Osteoporosis would appear to constitute the major problem of bone loss. Dietary deficiency was not seen to be a contributory factor in the two groups studied, and the loss of bone was almost certainly secondary therefore, to failure to absorb if dietary factors are implicated. That the removal of part of the stomoch might lead to the removal of some element concerned with calcium homeostasis can be discounted since the patients with polya-gastrectomies did not differ significantly from patients who had had a vagotomy and gastro-jejunostomy. The important dietary constituent concerned with the loss of bone mass remains speculative. Failure to absorb calcium could be a contributory factor. However, the mean urinary calcium excretion measured as the calcium/creatinine clearance ratio tended to be higher than that seen in normal subjects on a free diet (Nordin and Smith, 1965) (normal range 0.03 to 0.28).

The urine calcium is known to fall on a low calcium diet (Nordin and Smith, 1965), and it would be logical to expect a low calcium excretion if the absorption of calcium was poor. The slightly higher values in the patients with gastric surgery could well be associated with the relatively high calcium intake, but the implication here is that the calcium absorption is adequate on their diet to maintain the urinary calcium level.

The measurement of calcium absorption in patients following gastric surgery by us (Shimmins, Smith, Aitken, Orr and Gillespie, 1970) using a calcium isotope did not show any evidence of poor absorption even in the patients with early osteomalacia, though the absorption was diminished in one patient with severe steatorrhoea and osteomalacia. The method employed in this study was that of the Occupancy Principle (Orr and Gillespie, 1968) and the calcium absorption was measured over a period of two weeks. Other studies of calcium absorption using ^{47}Ca in patients who had had gastric surgery showed them to have a higher calcium absorption on average than was seen in the normal subjects (Robinson, Gutteridge, Joplin, Belcher and Fraser, 1964). The use of techniques employed are open to suspicion that they do not necessarily measure the true absorption of dietary calcium, nonetheless, the balance of

evidence at present is that there is no demonstrable evidence in favour of diminished calcium absorption in patients following gastric surgery.

On the other hand, the results of Thompson et al (1966a and 1966b) show that vitamin D absorption may be diminished following gastric surgery. If the above arguments in favour of adequate calcium absorption following gastric surgery are acceptable then the further implication is that if there is diminished vitamin D absorption it is not of such a degree as to limit calcium absorption. It is generally accepted that vitamin D has a direct effect on bone and it is possible therefore that minor degrees of vitamin D deficiency may cause the net loss of bone by leading to a diminished rate of bone formation or by an increased rate of bone destruction. This explanation would fit the information which is available, but direct confirmation is obviously desirable.

Finally, it is evident that there is no difference between the two patient groups, in their dietary intake, biochemical values, or in the degree to which their X-ray indices of bone mass have been affected. It is evident that gastric surgery does affect calcium metabolism, but there is no reason to select one type of operation in preference to the other.

CHAPTER IX

THE EFFECTS OF CALCIUM SUPPLEMENTATION OF THE DIET ON BONE MASS IN WOMEN

Since osteoporosis is a condition in which the amount of bone present is diminished then clearly this must have arisen because the rate of bone destruction is greater than the rate of bone formation. This could arise from a fall of the bone formation, a rise in the bone destruction rate or a combination of these factors which results in the rate of bone formation being lower than that of destruction. These changes could in turn be due to some property of the osteoblasts or osteoclasts. Alternatively, the cause could lie in some factor controlling the metabolism of the cells such as the secretion of parathyroid hormone, calcitonin and oestrogens, or could result from exogenous factors such as an inadequate dietary intake of calcium or vitamin D.

Albright et al (1941a) suggested that the cause of osteoporosis was due to a fall in the rate of formation of bone matrix. More recent studies have supported the view that the bone formation rate is normal in subjects developing osteoporosis, but that the rate of bone destruction is increased. The latter view is supported

by the investigations in Chapter VI which do show a fall in the rate of bone formation, measured isotopically, with age, but as there is also a fall in the amount of bone present, the rate of formation per unit mass of bone is unchanged. The urinary hydroxyproline excretion increased with age, and as the output of urinary hydroxyproline correlated inversely with the balance, and directly with the bone destruction rate measured isotopically, this finding is in keeping with an increased bone destruction rate with age.

Low calcium diets, in the presence of adequate vitamin D, have been reported to produce osteoporosis in animals (Weiske, 1874; Pommer, 1925; Jaffe, Bodansky and Chandler, 1932; Bell, Cuthbertson and Orr, 1941; Schmidt, 1937). Jowsey and Gershon-Cohen (1964) repeated this work on cats and were able to demonstrate that the osteoporosis could be reversed by adding calcium to the diet. Positive calcium balances have been reported in patients when their diets have been supplemented with calcium (Owen et al, 1940; Anderson, 1950; Whedon, 1959; Harrison and Fraser, 1961; Nordin, 1962).

Since the object of therapy in osteoporosis is to increase the bone mass, the best parameter to measure would be the changes in mineral content of the bone. Until recently, the measurements of bone mineral content have been too crude to determine the relatively small change that occurs in bone mineral content even over periods of many months or years. However, with the development of the technique described in Appendix I, it has been possible to follow the bone mineral content changes in the third metacarpal of the right hand in patients who were referred to a special bone clinic because they were thought to be osteoporotic (Chapter VII). These patients have had bone mineral content measurements done at 6 monthly intervals from periods ranging from 18 to 54 months, and the results are reported below.

Procedure

All the patients had X-rays taken of their right hands under the conditions described in Appendix I with a standard aluminium stepwedge which was exposed at the same time on the X-ray plate. The patients investigated represent 94 of the 97 patients described in Chapter VII.

From the investigations in Chapter I, it is clear that there can be a fall in whole bone density without a significant fall in the total amount of bone present because of the relative rates of increase of the internal and external diameters of bone with age. Therefore, both the whole bone density (S.A.E.) and the total cortical mineral (T.C.M.) have been estimated in these patients. The patients in the study were given either calcium glycerophosphate in a dose of 2 gm t.i.d. (supplementary daily calcium 1.14 gm/day) or calcium Sandoz tabs. 1, t.i.d. (supplementary calcium 1.14 gm/day).

In a group of patients receiving therapy, such as calcium supplements over a long period of time, some assessment of the persistence with which the therapy was taken is necessary. Patients attended the clinic at 3 monthly intervals and the calcium supplements were issued through the Hospital Pharmacy Department to cover the period until their next visit. This, associated with the frequent visits to this clinic, helped to assess whether or not the tablets were being taken. The patient was allowed time to ask for further prescriptions as this usually implied that the tablets were finished and must

therefore have been taken regularly. If not, the patients were asked if they needed a further supply. They were also asked if they were taking their tablets regularly. It was felt that a reasonable estimate could be made of those patients who were taking their tablets regularly. Those patients who did not take their tablets regularly were excluded from the trial. X-rays were taken at 6-monthly intervals.

In every patient, the serum calcium, phosphorus, alkaline phosphatase and creatinine was estimated. In addition, the urinary calcium:creatinine ratio was estimated and the Phosphate Excretion Index (P.E.I.) was calculated. The questionnaire described in Appendix III was completed. Any patient found to have other conditions which might affect the bone density was excluded from the trial.

Patients studied

The patients studied were the female subjects referred to a special bone clinic because they were considered to be osteoporotic after examination of their X-rays by the referring physician or surgeon. They represent 94 of the 97 patients described in Chapter VII. No further attempt was made to select the patients except on the conditions described above. In all 94 patients were studied for periods of not less than 18 months and up to periods of 54 months. The normal subjects with whom they are compared are those described in Group I and Group III in Chapter I.

Results

Fig 141 shows the initial whole bone density (S.A.E.) of the patients studied in relation to the distribution of the S.A.E. in normal subjects. The patient group had a slightly lower mean S.A.E. than the normal subjects as can be seen in Fig 150 in which the S.A.E. percentile values are shown, but this difference does not reach statistical significance. The initial percentile value of the T.C.M. is shown in Fig 151. Again the values are slightly but not significantly lower than those found in the normal subjects.

Fig 142 is a histogram showing the age distribution of the patients studied. Eighty-seven percent of the patients lay between 50 and 80 years of age. The mean age of the patient group is 61 years.

The change in S.A.E. in relation to the time since starting therapy is considered in Fig 143. The S.A.E. in each patient at the time of starting the trial, and at 6 monthly intervals thereafter, is shown. There is a small fall in the S.A.E. over the period of the study. The mean rate of fall in the S.A.E. and the T.C.M. in the normal subjects of Group III with age is plotted in Figs 144 and 145. It is seen that the rate of fall varies with age. The mean rate of fall in the S.A.E. in the normal population increases with time from 34 to 62 years of age after which the rate of bone loss decreases with advancing age.

The mean rate of fall in the T.C.M. (Fig 145) reaches a value of -0.05 at 62 years and shows little change thereafter. The rate of fall in the S.A.E. and the T.C.M. is regressed against age in each of the patients studied, and the rate of

fall is plotted against the mean age of the patient during the period of therapy (Figs 144 and 145). There is no obvious difference between the groups so studied.

In order to investigate this further, all the patients who had a statistically significant rise or fall in the S.A.E. or the T.C.M. were re-examined (Figs 146 to 149). Three patients showed a significant rise in the S.A.E. and two a significant rise in the T.C.M.. Twenty-six patients showed a significant fall in the S.A.E. and 24 a significant fall in the T.C.M.. The number of patients showing a significant rise in the S.A.E. and the T.C.M. were small, but no specific feature in terms of initial S.A.E. or T.C.M. (Figs 146 and 148), marital status, parity, age at the menopause, social class, urinary calcium excretion or calcium absorption, distinguished these patients from the rest. The patients who showed a significant fall (Figs 146 and 148) again were not found to differ from the population as a whole when considered in relation to the factors reported above. In Figs 147 and 149, the patients who did not individually show any significant change in S.A.E. or T.C.M. are illustrated. Again they did not differ significantly in any way from the normal population. The group as a whole are examined further by estimating the percentile values in relation to the normal population and the results are illustrated in Figs 150 and 151. There was a small

fall in the percentile values of both the S.A.E. and the T.C.M. with time since starting therapy. When regressed against time since starting therapy, this fall was found not to be of significance (Table LIII). However, there was a significant fall in both the S.A.E. and the T.C.M. when the rate of change, measured as the difference from the initial value, was regressed against time on therapy (Table LIII). This means, that as a group, the patients treated with calcium supplements are showing a rate of fall in both whole bone density (S.A.E.) and total cortical mineral (T.C.M.), greater than the rate of fall seen in the normal population.

Discussion

The measurement used to detect differences in bone mass in the 94 patients studied while they were receiving calcium supplements, was made on the 3rd metacarpal. As shown in Chapter II, the mineral content of the metacarpal is related to the mineral content of the spine. The changes in bone mass with age are rather lower than those found in the mid radial shaft by Sorenson et al (1968) but show the same general pattern.

Conrad Johnston, Smith, Yu and Deiss (1968) described a more rapid fall in the lower end of radius, which has a significant amount of trabecular bone, than in the mid shaft. It is evident that the measurements in one bone will not accurately predict the rates of change in all bones. However, the pattern of change is similar. The metacarpal measurement is a convenient and accurate one (Chapter I, Appendix II), and there is no reason to believe that it does not predict similar changes in other parts of the skeleton, even if the rates differ.

The patients studied have been compared with the normal population of women, as described in Chapter I. They had a slightly lower whole bone density (S.A.E.) and total cortical mineral per unit length of bone (T.C.M.) than the normal population, but this was not statistically significant. The rate of fall of bone mass in the normal population varies with age as can be seen from Figs 144 and 145 , but the standard deviation does not differ significantly within the different age groups. The 94 patients studied did not differ from the 152 normal subjects of Group I in terms of social class, age at onset of the menopause, parity, breast-feeding, dietary intake of calcium, phosphorus, protein or vitamin D, urinary calcium excretion or calcium absorption. The 152 normal subjects of Group I are described in Chapters I and VII. The normal subjects of both Groups I and III were recruited in the same way from the relatives and friends of patients in general medical wards. None of the patients had any metabolic bone disease that might have affected their bone X-ray measurements.

The changes in the whole bone density (S.A.E.) of the patients during the period of therapy are examined at 6 monthly intervals for the whole patient population (Fig 143). There was a small fall in the mean of each measurement over the period of the study. However, since the rates of change in the S.A.E. and the T.C.M. vary with time (Figs 144 and 145), the rates of change with time as measured by the gradient of the fall are illustrated for each patient at her mean age over the period of study in the same figures. From inspection it is evident that though there is a wide scatter, the mean rate of change does not differ noticeably from that found in the population as a whole.

A small number of patients did show a significant improvement in bone mass. The numbers are too small to permit statistical analysis, but when compared with that proportion of the population which showed no significant change or with the normal population of Groups I or III, no obvious differences were observed. A large number of patients showed a significant fall in the S.A.E. and the T.C.M. with age. Again these patients

cannot be distinguished from those who had shown no change or from the normal population at the age of onset of the menopause, marital status, social class, parity, length of time of breast-feeding, dietary intake of calcium, phosphorus, protein or vitamin D, urinary calcium excretion or calcium absorption.

However, the normal population shows a significant fall in both the S.A.E. and the T.C.M. with age. Even if calcium can reduce the rate of fall below that of the normal population, it will be of benefit. Any change producing a more or less rapid fall in the patient group when compared with the normal subjects will be evident in a changing percentile value. These are illustrated in Figs 150 and 151, and regressed against time in Table LIII. The percentile value shows no significant change, and evidently the patients are showing a fall in the S.A.E. and the T.C.M. which is close to that of the normal population. However, when the change in percentile value is measured for each patient as the change from the starting value, then there is a significant fall in both S.A.E. and T.C.M. percentile with time on therapy. This fall means that the patients are showing a fall in both whole bone density and total cortical mineral per unit length of bone which is greater than that in the normal population.

This must mean either that the patient population is different from the normal population, or that the calcium supplement is causing an accelerated rate of bone loss. The comparison between the normal and patient groups have not brought to light any significant difference between them. The evidence therefore is in keeping with the therapy being the cause of the accelerated bone loss in the patient group.

This is at variance with the positive calcium balances with calcium supplementation which have been reported so often, and with the evidence shown in Chapter VI. This evidence does exist, however, and it would be superficial to ignore it without further investigation. Logically, all the balance data reported must regularly and persistently overestimate balance studies when calcium supplements are added, or else the positive balance must be relatively short-lived in spite of claims to the contrary. This question is examined further in the next chapter.

CHAPTER X

THE EFFECTS OF CALCIUM SUPPLEMENTS ON CALCIUM BALANCE, FAECAL CALCIUM AND FAECAL FAT

Introduction

It is evident from the studies in Chapter IX that giving calcium supplements for periods up to 54 months has not resulted in a significant increase in whole bone density (S.A.E.) or total cortical mineral (T.C.M.) measured on the 3rd metacarpal of the right hand. There has in fact been a fall in the S.A.E. and the T.C.M. which is significantly greater than that found in the normal female population of the same age. This is at variance with the reported positive balance by a number of authors (Owen et al, 1940; Anderson, 1950; Whedon, 1959; Harrison and Fraser, 1961; Nordin, 1962), and with the fall in urinary hydroxyproline and diminished bone destruction rate measured isotopically as shown in Chapter VI. The studies in Chapter VII also show little evidence of a relation between dietary intake and the X-ray indices once age and weight have been allowed for. It is evident therefore that either the methods are inadequate or else the positive balance achieved is only a temporary one. In view of these contradictory findings, the effects of calcium supplementation have been investigated further, and the results are reported below.

Materials and methods

Five female and one male subject were investigated. These patients were admitted to a metabolic unit. The age, sex and diagnosis of each patient is shown in Table LIV. Also shown is the S.A.E. (whole bone density). All the patients had a value below 28 which is used as a quantitative definition of osteoporosis (Chapter II). The percentile value for the S.A.E. in each patient, which indicates their whole bone density relative to the normal population at their age is also shown. These patients remained on a basic diet with a fixed intake of calcium and fat, and this was supplemented firstly as organic salts, then in the form of dried skimmed milk. The details of the calcium and fat intake for each patient during each period of study is shown in Table LIV. The calcium intake on the basic diet ranged from 0.233 gm/day to 0.733 gm/day. The fat intake on the basic diet ranged from 32.9 to 65.9 gm/day. Each patient was allowed one week to equilibrate on the basic diet before starting the investigation, and was first investigated on this basic diet for 4 to 6 weeks (Period A). The calcium intake was increased during the next 4 weeks (Period B) by giving calcium glycerophosphate to patients 1 to 5

and calcium gluconogalactogluconate to patient 6. This increased the calcium intake by 1.14 gm/day. During the next 4 weeks (Period C), the calcium intake was raised by 1.24 gm/day above the basic level by giving the equivalent of $1\frac{1}{2}$ pints of dried skimmed milk daily to patients 1 to 5. The fat content remained unchanged during Period B. There was a very small rise in fat intake when the dried skimmed milk was added to the diet, the increase being 0.3 gm/day during Period C.

Faeces and urine was collected in 7 day aliquots. The faeces was collected directly into homogeniser cans, homogenised and aliquots taken for the estimation of fat, bile acids and calcium. The bile acids were extracted from freeze-dried faeces with 95% aqueous ethanol under nitrogen for 6 hours. The bile acids were quantitated by thin layer chromatography (Mitchell and Diver, 1967). Dietary and faecal calcium was measured after ashing by titrating with EDTA ammonium purpurate being used as an indicator.

Results

In Table LV, the calcium balance is shown in patients 1 to 5. During the Periods A, B and C, the urinary hydroxyproline excretion is also shown. The balances are taken as the mean of 4-6 weeks during the control period, and as the mean of the last three periods in each of the Periods B and C, so that one week was allowed for equilibration. As can be seen, all the patients were in negative calcium balance on the initial intakes (Period A). The balance improved during Periods B and C, a positive balance being achieved in all the patients during Period C. The urinary hydroxyproline excretion decreased during Periods B and C, the lowest values occurring during Period C.

The change in excretion of faecal calcium, faecal fat and the faecal dry weight from the first week of the basal period is shown in Fig 152. The mean and standard error for each weekly period is indicated. In Table LVI, the mean faecal dry weight, acid fat, total fat and calcium for the periods A, B and C is shown. There is a significant increase in the faecal calcium ($P < 0.001$), the faecal acid fat ($P < 0.02$), total fat ($P < 0.05$) and faecal dry weight ($P < 0.01$) during period B (i.e. during calcium glycerophosphate supplementation of the diet). The substitution of dried skimmed milk for calcium glycerophosphate did not affect the faecal fat, dry weight or calcium compared with period B. The faecal dry weight and calcium remained significantly above the level of excretion during the period on the basic diet. None of the patients complained of any gastro-intestinal symptoms or had any diarrhoea during calcium supplementation of the diet (Table LVI).

In Fig 153, the excretion of faecal bile acids is shown. There is a significant increase in the output of deoxycholic and lithocholic acid and also an increase in the total bile acid output. The increase became apparent after the end of the first week and continued throughout the period of calcium supplementation (i.e. during both periods B and C) (Table XLVII).

Discussion

The total faecal calcium, faecal fat and faecal dry weight increased in all the patients studied during supplementation of their diets. This was accompanied by an increase in the excretion of bile acids. It is possible that the calcium in the forms tested combined with the bile acids to produce insoluble salts, which in turn led to decreased emulsification and digestion of fat, though the formation of insoluble calcium soaps of fatty acids is perhaps more important.

It is interesting to note that the dried skimmed milk appeared to have a smaller effect on the faecal fat excretion and the excretion of bile acids than the organic salts. This occurred in spite of the amount of calcium given which was slightly greater (by 100 mg/day) and the dietary fat also increased slightly (by 0.3 gm/day). The balance improved in period B compared with period A, but it was during period C, on skimmed milk, that the balance was most improved. Furthermore, the urinary hydroxyproline excretion fell during period B but was lowest during the period of skimmed milk supplementation of the diet.

These results are in keeping with the positive calcium balance produced by supplementary calcium reported by Owen et al (1940); Anderson (1950); Whedon (1959); Harrison and Fraser, (1961); Nordin (1962). However, as the bone mass continues to fall while patients were taking calcium supplements (Chapter IX),

it is clear that the positive balance, the fall in urinary hydroxyproline and the decreased bone resorption rate reported in Chapter VI and by the above authors cannot be more than only a very temporary one.

There are now several reports in the literature that calcium supplementation of the diet interferes with the absorption of fat in both experimental animals and in man. Bassett, Keutmann, Hyde, van Alstine and Russ (1939) reported that faecal fat excretion increased in patients with steatorrhoea who were given calcium supplements. Lutwak, Laster, Gitelman, Fox, Whedon, Wolfe and Woodson (1964) reported this effect in children. Weiner and Lutwak (1963) had previously shown a similar effect on young rats. Kane, Lovelace and McCay (1949) found that fat absorption was decreased in old rats and that absorption was further depressed by the addition of calcium to their diet.

The increased excretion of faecal fat would suggest the reason for the decrease in calcium balance. In Chapter II and Chapter VIII, it was shown that patients with steatorrhoea who had had gastric surgery in the absence of osteomalacia showed an increased bone loss when compared with normal subjects of their own age. The mean rise in faecal fat excretion from

2.31 gm to 3.69 gm is not large, but over a long period of time could be important.

It is evident that a positive balance is only possible when the bone formation rate is greater than the bone destruction rate, and that a negative balance will be associated with a bone destruction rate greater than a bone formation rate. The balance could be the primary cause in which case decreased calcium absorption or increased calcium loss could be the important factor. Alternatively, the fault could lie in the bone, the negative calcium balance resulting from the bone destruction rate being higher than that of the bone formation rate.

The information available would suggest that a temporary positive balance can be achieved, but that in time the balance must become negative in spite of continued supplementation of the diet. The time sequence of events would be in keeping with the loss of some nutrient which would be responsible for either decreased calcium absorption or a relative increase in bone destruction. Furthermore, there is evidence of decreased calcium absorption with age (Chapter VII).

Vitamin D deficiency of a mild degree could be the factor involved, and the results so far available would suggest that a trial of vitamin D with or without calcium supplements would be worthwhile.

However, it is extremely unlikely that this simple explanation will contribute more than a small part of the answer to the problem. The important factor here is the difference between male and female subjects - bone loss is a relatively unimportant factor in males, though it is unlikely that there is a major difference in the environment of male and female subjects. It would seem much more likely that the major cause of bone loss in women is genetic, and is an expression in some form of the X chromosome. This does not mean that the steady loss of bone with age cannot be prevented, but the evidence against simple calcium supplementation of the diet as being useful is now very strong.

CONCLUSIONS

CONCLUSIONS

The Conclusions are considered under the same headings that are used to define the problems in the Introduction. To summarise:

1) The problems of definition of osteoporosis are considered in Chapters I, II and III. The factors associated with variations in bone mass in the normal male and female population are examined in Chapter I. The variations from the normal due to pre-disposing pathological states are examined in Chapter II. The relation between bone mass and fracture incidence are considered in Chapter III. The quantitative definition of osteoporosis is then considered in the light of these investigations.

2) Factors affecting calcium balance are examined in Chapters IV and V. In Chapter IV, the isotopic measurement of calcium absorption is discussed. Chapter V is concerned with the control of urinary calcium excretion.

3) Bone formation and destruction rates are considered in Chapter VI which is divided into two Parts. Part I is concerned with the measurement of the bone formation rate and the effects of diet and age on bone mass. Part II is concerned with the measurement of bone destruction rate using isotopes, and urinary hydroxyproline excretion, and the related changes in urinary hydroxyproline excretion with age.

4) In Chapters VII to X, the role of the dietary intake of calcium, phosphorus, protein and vitamin D in relation to bone mass, the absorption of calcium, urinary calcium excretion and the effects of calcium supplementation of the diet are described. Chapter VII is concerned with the relation between calcium, phosphorus, protein and vitamin D intake and bone mass, and the variation in these in relation to age and sex. Chapter VIII is a study of the changes in the calcium and phosphorus intake and excretion and their relation to bone mass in patients who have undergone gastric surgery. The effects of calcium supplementation of the diet on the bone mass of women is examined in Chapter IX. In Chapter X, the effects of calcium supplementation of the diet on faecal bile acids and faecal fat are reported.

1) The problems of definition

As discussed in Chapter I, the skeletal mass varies throughout life in both men and women. The rates of change in bone mass depend upon the type of measurement used, the age and sex of the population examined. The measurements used in this study are the whole bone densities (measured as the standardised aluminium equivalent - S.A.E.), the cortical density (λ), the total cortical mineral per unit length (T.C.M.) of the 3rd metacarpal, the X-ray indices of the metacarpal (M.I.), femoral index (F.I.), Spinal Index (S.I.), and the Relative Vertebral Density (R.V.D.). The reason for these differences arise because the external and internal diameters of the long bones change at different rates throughout life, and because of variations in the density of bone as a tissue with time. The highest rates of change are seen in the S.A.E. (whole bone density) and the X-ray indices of the metacarpal and femur. The whole bone density rises from the age of 5 to 35 years in both men and women and falls thereafter, the rate of fall being twice as fast in women compared with men between 50 and 60 years of age. The X-ray indices (M.I. and F.I.) show a very similar

pattern of change. The cortical density rises rapidly up to the age of 20 years in both men and women. In the male it continues to rise slowly throughout life, thereafter. In the female, there is a slow rise until the age of 40 years and a small fall occurs thereafter up to the age of 75 years. The T.C.M. rises rapidly in men and women up to the age of 21 and in the males then shows a very slight fall. In women, there is a much more pronounced fall after the age of 50. The Spinal Index falls very slowly with age in women, but not significantly in men. The R.V.D. falls slightly in the male, but to a much greater extent in the female. The rates of change in all the measurements used, except for the cortical density, are accelerated in women at the time of the menopause. Social class does not affect bone mass.

The largest surveys have been carried out using the densitometric measurement of the 3rd metacarpal in normal male and female subjects, and the relative rates of change between the sexes have been examined in more detail. After 35 years of age, the whole bone density shows the greatest fall in both the male and female subjects. The relative rates of change, however, are greatest in the T.C.M. which shows a rate of change eight times greater in the female than in the male. For the S.A.E. the rates of change are only two to one.

In Chapter II, the bone mass in a variety of pathological conditions has been examined. From this study, it is clear that if no allowance is made for size, then it is impossible to compare the effects of the pathological conditions affecting the individual. The measurements which correct for size are the whole bone density, Metacarpal Index, Femoral Index and Spinal Index. Cortical density and Relative Vertebral Density are, of course, independent of size. In Chapter II, the effects of the pathological states on the S.A.E. and λ are described. To define the bone status of any individual in relation to the normal population, it is also necessary to determine the percentile value of the S.A.E. and λ and these too have been considered.

Female patients referred to a special Bone clinic because they were thought to be osteoporotic by the referring clinician are found not to differ significantly from the normal subjects of their own age in either whole bone density or cortical density. Men referred in this way, however, were found to be significantly below the mean for their age when compared with the normal male population. The reason

for this is that only a small change in the S.A.E. occurs in normal men with age, while a much greater change occurs in women. The experienced clinician estimates the bone mass in his mind against the idealised picture of the young healthy adult. In the male, he will select and refer patients who, though within the normal range, will have a bone mass below the mean. In the female subject, however, he is not able to make allowance for the marked change with age, and the patients referred are those over 50 years who presented with symptoms, but who otherwise do not differ from the normal population.

The whole bone density is lowered in the patient who has had gastric surgery, but the cortical density is normal in the vast majority. Both the whole bone density and cortical density tends to be low in patients with steatorrhoea, osteomalacia and hyperparathyroidism.

Female patients with fracture of the femoral neck do not differ significantly from the normal population in terms of S.A.E. or λ. They are merely subjects from the older normal population.

However, virtually all have an S.A.E. below 28. This is the 5 percentile value at age 35 years of the normal population when the whole bone density (S.A.E.) is at its peak. This is in agreement with the relation between whole bone density and spinal biconcavity in women. Measurement of the Spinal Index shows a significant increase in the incidence of spinal biconcavity when the S.A.E. falls below 28. The degree of spinal biconcavity is clearly in no way related to the percentile value. This means that the development of spinal biconcavity is a function of the total bone present, and not a function of the amount of bone in relation to that present in normal subjects of the same age. In Chapter III, the incidence of fracture is seen to increase with age in both men and women. The bone mass decreases with age in both men and women, when measured either as the A.E., S.A.E., T.C.M., or S.T.C.M.. Clearly, the rate of change of any of these measurements can therefore be correlated with the rate of increase of fracture. However, it is only the rate of change in the total cortical mineral per unit length which correlates with the rate of increase of fracture incidence in both men and women. The assessment of the possibility of any individual patient sustaining a fracture requires the T.C.M. to be known.

Clearly, from the foregoing, the definition of osteoporosis quantitatively, requires that several parameters of bone mass must be known and reported.

These are:

1) The cortical density which is nearly constant between 20 and 75 years. At lower ages λ is affected by the higher proportion of young bone present which has a lower density. At the upper end of the age range λ again tends to fall in women because of increased cortical porosity which is characteristic of severe osteoporosis. The measurement of λ will help to differentiate osteomalacia from osteoporosis.

2) In order to compare individuals, allowance must be made for the difference in size in individuals. If this is not done a large individual will have a higher value for bone mass than a small one, even if the former is in fact osteoporotic and the latter is normal. The values independent of size which have been measured are: λ (cortical density), S.A.E. (whole bone density) and S.T.C.M. (total cortical mineral divided by the square of the external radius). All these measurements refer to the 3rd metacarpal. The X-ray indices M.I., F.I., S.I., and the Relative Vertebral Density are also independent of size.

3) Apart from bone pain, the increased risk of fracture is the most important consequence of diminished bone mass. In order to determine the risk of fracture some measure corresponding to the total cortical mineral per unit length of bone ($T_{\cdot}C_{\cdot}M_{\cdot}$) must be determined.

4) In order to attribute a value to each of these measurements in relation to the normal population, the percentile value for each measurement must also be known.

Clearly no single measurement will completely define osteoporosis which should be considered in relation to all the above parameters. If these are determined on several occasions, over a sufficient interval of time, it is possible to determine quantitatively how the bone of any patient is changed in relation to the normal population.

Finally, though no single measurement adequately defines osteoporosis, it is useful to be able to decide on one measurement whether any one patient should be subjected to the rigours of further investigation and treatment.

Since spinal biconcavity increases significantly when the S.A.E. falls below 28, this is a useful value which can be taken as a practical dividing line below which any therapy might be instituted.

2) Factors affecting calcium balance

In Chapter IV, a double calcium isotope method of measuring calcium absorption is compared with the plasma value obtained 2 hours after the oral administration of the isotope. The reason for this investigation is that the two hour plasma value is clearly dependent on a number of different factors. It will be affected by the rate of absorption of the isotope, the rate of mixing within the rapidly exchanging pool, the size of this pool, the rate of loss into bone, the rate of loss in the urine and minor losses back into the gut as endogenous secretion. By injecting an intravenous tracer at the time of the orally administered isotope and comparing the integrated specific activities in the plasma of the isotopes administered by both routes, the rate of transfer of isotope from gut to blood can be determined. This technique requires only one assumption and that is that the orally administered isotope once absorbed into the blood behaves in the same way as the intravenously administered isotope. This condition is satisfied as discussed in Chapter IV. The value so obtained

is, of course, quite independent of the rates of loss of the isotope from the plasma pool. The two hour plasma blood value does correlate with the value obtained by the double isotope technique.

The reason why this investigation was carried out was to determine if the two hour plasma value was a reasonable estimation of calcium absorption in the normal and 'osteoporotic' populations studied, since it is quite impracticable to do the more complex procedure in large numbers. This investigation justifies the use of the two hour plasma level, corrected for body weight, in populations in whom very widely differing rates of disappearance of isotope from the plasma pool do not occur - e.g. in Paget's disease. The results obtained in the populations studied are discussed in Chapter VII.

The calcium balance can also be affected by urinary calcium losses. The factors affecting urinary calcium losses are examined in Chapter V.

Calcium intake affects urinary calcium loss. Low calcium intake leads to a fall in urinary calcium excretion, and high calcium intake to increased urinary calcium. The changes are the result of increased calcium intake causing a small but significant rise in the plasma ultrafilterable calcium which in turn causes a rise in the filtered load. The change in plasma calcium correlates with the urinary calcium loss. These small changes in plasma calcium do not produce any significant change in the activity of the parathyroid glands.

A raised phosphorus intake causes a fall in urinary calcium. This is mediated through a fall in plasma ultrafilterable calcium which is in turn associated with a rise in the plasma phosphorus. Part of the fall in plasma ultrafilterable calcium could be associated with diminished calcium absorption due to complexing of the calcium with the additional phosphorus in the gut. Unlike the changes in ultrafilterable calcium produced by low and high calcium intake, changes in the intake of phosphorus clearly affects parathyroid activity. This must mean that the fall in plasma calcium induced by high phosphate

feeding causes a fall in the ionic calcium while low calcium intakes cause a fall in the complexed ultrafilterable fraction. Urinary calcium falls during high phosphate feeding. This is due partly to the fall in the filtered load. However, increased parathyroid activity also causes an increase in the reabsorption of calcium by the renal tubule (p. 137), and part of the fall in urinary calcium could be due to the parathyroid stimulation due to the high phosphate diet. In hypoparathyroidism, the calcium reabsorption is diminished (p. 138). Clearly, a high phosphate diet could affect the bone mass by stimulating the parathyroids which affect the rates of bone formation and destruction.

3) Bone formation and destruction rates

The rates of formation and destruction of bone are examined in Parts I and II of Chapter VI. Before any conclusions can be drawn from the measured formation and destruction rates using isotopes, an examination of these techniques is necessary. From the quantitative auto-and micro-radiographic studies of Marshall et al (1959a,b,c,d), Rowland (1963), and Lloyd (1965), it is evident that the isotope is incorporated into bone both by active bone formation, when there is net accretion of calcium, and by iso-or heteroionic exchange. Exchange, especially at bone surfaces, occurs rapidly and contributes to the fast exchanging calcium pool which has a value of about 5 gm of calcium. Slower exchange occurs into inactive old bone, and the magnitude of this pool cannot be measured by the kinetic studies so far used in man. The auto-and micro-radiographic studies mentioned above indicate that between 10 and 50 percent of isotope may be taken up in this way. Much discussion in the past decade has also been concerned with the amount of isotope which is lost from bone due to bone resorption.

These problems have been studied by us and are reported elsewhere (Shimmins and Smith, 1966; Shimmins, Allison, Smith and Speirs, 1967; Smith, Speirs and Shimmins, 1967; Smith, Speirs and Shimmins, 1968; Shimmins and Smith, 1970; Shimmins, Lee, Smith and Lucy, 1970). These studies are concerned with the problem of exchange, the return of isotope due to bone resorption, and a comparison of auto-and micro-radiographic studies in dogs with tetracycline uptake estimation of bone formation rate. These studies have shown:

i) The return of isotope from the skeleton for periods up to 100 days in the normal adult and 'osteoporotic' subject is almost entirely due to exchange and only 2 to 3 percent of the isotope returns to the plasma because of bone resorption.

ii) The most accurate measurement of isotope uptake by bone (i.e. both by exchange and due to accretion) can be derived from long-term studies up to 100 days. This is necessary so as to eliminate the effects of the rapidly exchanging pool on the estimations, which dominate the measurements up to about 40 days.

iii) This is done by estimating the true rate of loss of isotope from bone and allowing for this in the calculation of true bone uptake. The reason for this is that the whole body retention is not a measure of the bone retention because isotope returning from bone acts as a fresh injection into the plasma. Part of the isotope only is lost from the body, the rest returns into the bone. The whole body retention measurements thus underestimates the true rate of loss of isotope from bone.

iv) Since it is impractical to study whole body retention over such long periods of time if a large number of patients are to be examined, the correct value so obtained is used to determine the best time to estimate the calculation of the isotope uptake by the method of Bauer, Carlsson and Lindquist (1957). The values so obtained indicates that the value between 5 and 10 days by this method is the best time to estimate the bone uptake of ^{45}Ca or ^{47}Ca, and between 7 and 14 days for ^{85}Sr.

v) Though the values so obtained with ^{85}Sr give a lower value than the values obtained with ^{45}Ca or ^{47}Ca, the strontium values do correlate with the values obtained with calcium (p. 159).

vi) In the dog, a correlation between the values obtained by isotopes of calcium and tetracycline labelling was found (Shimmins, Wood, Lee and Smith, 1970). Moreover, it was shown that with age there was a fall in bone formation rate which was matched by a corresponding fall in the value of the isotope taken up by exchange.

If these results can be extrapolated to man in the normal and 'osteoporotic' range, then the rate of bone formation can be seen to fall significantly with age in women. However, there is a corresponding decline in the bone mass with age which means that the rate of bone formation per unit mass of bone remains constant.

The rate of bone destruction falls on calcium supplements measured isotopically. This fall correlates inversely with the change in balance, and directly with the urinary hydroxyproline excretion. Furthermore, in Part II of Chapter VI, it is shown that the urinary hydroxyproline creatinine excretion rises with age. The reason for believing that the major part of the urinary hydroxyproline excretion comes from bone destruction has been discussed. These findings are in keeping with the view that the bone loss in women is due to an increased rate of bone destruction with age. These results are in agreement with those reported by Jowsey (1966) who used a quantitative microradiographic technique to determine bone formation and destruction rates in normal and 'osteoporotic' subjects.

4) The role of dietary intake of calcium, phosphorus, protein and vitamin D in relation to bone mass and age; the absorption and urinary excretion of calcium; the role of calcium supplementation of the diet

The dietary intake of calcium, phosphorus, protein and vitamin D in the normal male and female population in relation to bone mass is reported in Chapter VII. Three groups of subjects have been studied. The first was a group consisting of 152 normal women who were recruited from the relatives and friends of patients in the general medical wards of the Western Infirmary, Glasgow. The second group was composed of 97 women referred to a special Bone clinic because they were thought to be osteoporotic by the referring clinician. The third group was composed of 75 normal male subjects recruited in the same way. Each subject had a full dietary history taken. The calculated results agreed with the results obtained by careful weighing out of the diet over one week and are reported elsewhere (Harrison and Nordin).

The X-ray indices of the normal female subjects are compared with those of the 97 patients who were considered to be osteoporotic. It was found that though the patient group had a low mean X-ray score than the normal group, this was because they were, on average, older. When the X-ray indices of the normal and patient groups were

compared by decades, no significant difference was found between the two groups. A higher proportion of the patient group had had an artificial menopause and there was a higher incidence of backache and fracture in the patient group. This is not surprising as these were the main reasons for the patients being referred to the clinic, apart from the appearance of the bone X-rays. The two groups were otherwise very similar. The average age of the onset of the menopause was virtually identical. They showed the same distribution of the site of backache, though backache in neither group correlated with the degree of osteoporosis as judged from the X-ray indices. The parity of each group by decades showed no difference. They had breast-fed their infants for the same length of time. The social classes were equally represented in both groups. The dietary intake of calcium, phosphorus, protein and vitamin D was significantly lower in the patient group, but they were older and lighter. When the dietary intake was divided by body weight and the two groups were studied by decades, no significant difference between them was apparent. The calcium absorption and urinary calcium excretion again does not differ significantly in the patients as compared with the normal controls.

The striking feature in fact is the similarity between the two groups, even though they were selected in completely different ways. It is also evident that age is a major factor governing the development of osteoporosis. For this reason the two groups were combined and the effects of age studied. There is a significant fall in the X-ray indices and the Relative Vertebral Density with age. There is also a significant fall in the dietary intake of calcium, phosphorus, protein and vitamin D with age, the least significant change being the fall in calcium intake. There is a significant fall in the absorption of calcium with age. There is no change in the urinary calcium excretion with age. The fall in dietary intake and absorption with age, along with the fact that there is no corresponding fall in the urinary calcium excretion, is sufficient to account for the small negative calcium balance, which would be required to produce the measured fall in bone mass with age.

There is a direct relation between the dietary intake of calcium, phosphorus, protein and vitamin D and the X-ray indices, though the significance is considerably diminished when the dietary intake is divided by body weight. Since

the most important factor is age, this was taken into account by comparing the intake of those subjects with high and low X-ray indices and Relative Vertebral Densities by decades between 30 and 70 years of age. The results are illustrated in Figs 100 to 111. The dietary intakes have been considered both corrected and uncorrected for body weight. There are some significant differences in intake between those with high and low intake, but most of these are no longer apparent when the intake is divided by body weight. However, a significantly lower intake of protein, phosphorus and vitamin D persists in those patients with a low R.V.D. between 51 and 60 years of age. There was no significant difference in calcium intake. The same patients do not show this difference in the Metacarpal, Femoral or Spinal Indices. It is at this time of life in women that the rate of bone loss is accelerating. These differences do apply in the main to the trabecular bone of the spine. However, in view of the fact that no differences were found in the other measurements or any consistently different intakes at any other decade, these results should be interpreted with some caution.

Male subjects show a relatively slight fall in bone mass with age. This is especially true of the T.C.M. which falls by only 4% between 40 and 70 years. The male subjects have a significantly greater amount of bone than female subjects as measured by the T.C.M.. However, if an allowance for size is made, then the difference between them only becomes apparent after 50 years of age for the S.A.E., and after 60 years for the M.I.. The male subjects have a higher intake of calcium, phosphorus, protein and vitamin D than the female subjects. The males are heavier than the females, and when the dietary intake is divided by the body weight and then compared by decades, no significant difference is found between the sexes. The male subjects also show a small fall in dietary intake with age, but this does not reach statistical significance. The male subjects showed no fall in calcium absorption with age, and when the two hour isotope level indicating calcium absorption was corrected for body weight, they have a better calcium absorption than the female subjects throughout the age range 30 to 70 years. The calcium excretion measured as

The calcium:creatinine ratio showed no significant fall with age in the male subjects. The urinary calcium excretion in the male was lower than that in the female at each decade between the 4th and the 7th though it does not reach statistical significance **except in the 4th and 5th decades.**

To summarise, the male subjects do not show the same marked fall in bone mass with age which is seen in women. Furthermore, calcium absorption is significantly greater in the male throughout life, and there is no fall in calcium absorption with age. There is a small but not significant fall in dietary intake with age. In the female the calcium absorption falls with age as does the dietary intake. Neither sex shows a significant fall in urinary calcium loss with age. It is evident that these differences will explain the greater negative balance in women. It is, of course, not possible to say whether these differences cause the more marked fall in bone mass in the female, or are merely the effect of some other change which is responsible for both the negative balance and the bone loss.

In Chapter VIII, the changes in bone mass of patients who have undergone gastric surgery are examined. The relation of the change to dietary intake of calcium and phosphorus, the effects of age and the type of operation are also examined. Two groups are described. One group had undergone Polya-gastrectomy, the other vagotomy with gastro-jejunostomy. The two groups were otherwise very similar in age, weight and time since operation was performed. The intake of calcium and phosphorus did not differ significantly between the two groups. However, the intake of calcium and phosphorus was higher in the patient group than in the normal subjects when considered by decades, especially when divided by body weight. The two patient groups did not differ significantly in their bone mass as measured by X-rays, but there is a greater fall in the bone mass with age than was seen in the normal male subjects.

The urinary calcium excretion, as measured by the calcium:creatinine ratio, was within the normal range for the patients. They tended to have a higher phosphate excretion than the normal subjects, but this appears to be the result of the relatively high phosphate intake. The phosphate excretion returned to normal in all the patients on re-estimation after an overnight fast, except in one patient in early renal failure and in patients with osteomalacia. Only two of the patients had convincing evidence of osteomalacia, though five further patients with slightly abnormal results failed to re-attend for further investigation.

The calcium absorption was not tested in these patients, but the evidence from the literature (Shimmins et al, 1970; Robinson et al, 1964) is that calcium absorption measured by isotopic techniques is normal or high in patients who had undergone gastric surgery. Only in patients with severe steatorrhoea does calcium absorption show evidence of diminition following gastric surgery.

The effect of surgery is not related to the time since operation. The fall in bone mass in the older patient compared with the normal subjects therefore clearly indicates that the older the patient the less able he is to adapt to the effects of gastric surgery. Clearly the fall in bone mass is not related to removal of part of the stomach as both the patients with Polya-gastrectomy and vagotomy with gastro-jejunostomy show the same degree of bone loss. Unless the older patients represent a metabolically different group from the younger patients, the diminished bone mass must be the result of the operation. The operation must have caused either the diminished absorption of some substance other than calcium or it must result in some change such as the endocrine factors controlling bone formation and destruction. While the latter possibility cannot be excluded, the failure of absorption of some other important constituent of the diet would appear to be the more likely cause. This is unlikely to be dimished phosphate absorption from the evidence that urinary phosphate absorption tends to be raised in these

subjects on their home intakes, and returns to normal on fasting. Failure of protein absorption could be a contributory factor but the bulk of the evidence would favour vitamin D as being equally or more important. The diminished absorption suggested by the work of Thompson et al (1966a,b), and the lowered serum anti-rachitic activity reported by Smith et al (1964) suggests that a relatively small deficiency of vitamin D may contribute to the development of osteoporosis through its effects on bone formation and destruction rates. There is the further evidence from Chapter VII that calcium absorption diminishes with age in women in whom the bone mass is also falling. However, Rose (1964) showed that vitamin D can also cause a significant rise in urinary calcium loss so that little or no benefit in calcium balance results. The implications are either that the dose of vitamin D is critical or else that age may be the dominating factor.

Although the evidence considered above is not in favour of calcium deficiency in the diet being a contributory factor in the development of osteoporosis with age, this does not exclude the possibility that calcium supplements might arrest or reverse the process. This has been examined in Chapter IX. The effects on bone mass measured as the whole bone density (S.A.E.) or total cortical mineral per unit length (T.C.M.) are described in 94 patients treated with either calcium glycerophosphate or calcium in a dose of just over a gram per day. The calcium supplements are additional to their normal dietary calcium intake. The patients were studied over a period of 18 to 54 months. They did not differ significantly from the normal subjects of their own age in terms of dietary intake of calcium, phosphorus, protein or vitamin D, calcium absorption, urinary calcium excretion, social class, mean age at the onset of the menopause, breast-feeding, parity or in their bone mass measured as the S.A.E. and T.C.M.

The majority of the patients showed a continuing fall in the S.A.E. and the T.C.M. while on therapy. Furthermore, when the rate of fall was considered in relation to the rate of fall in the normal population of the same age, the patient group were observed as having a fall in the S.A.E. and the T.C.M. which occurred significantly faster than was seen in the normal subjects. Clearly, though a very few patients appear to benefit from calcium supplementation of the diet, the majority are adversely affected.

This evidence is at variance with the positive balance produced by calcium supplements and reported by a large number of researchers over the past decade, and as reported in Chapter VI. The effects of calcium supplementation of the diet is examined further in Chapter X. The addition of calcium supplements to the diet of 6 patients resulted in a significant increase in the faecal excretion of calcium, bile acids and fat, the faecal output of these substances increasing gradually over 3 to 4 weeks. Increased faecal fat excretion has also been reported in patients with steatorrhoea who were given additional calcium (Bassett, Keutmann, Hyde, van Alstine and Russ, 1939) in children (Lutwak, Laster, Gitelman, Fox, Whedon, Wolfe and Woodson, 1964), and (Werner and Lutwak, 1963) in young rats. Kane, Lovelace and McCay (1949)

reported decreased fat absorption in old rats which decreased further on the addition of calcium to the diet.

Clearly this may be an important factor in long-term therapy. Patients with steatorrhoea and following gastric surgery, when the faecal fat excretion is known to increase, showed an accelerated bone loss. The precise reason for this requires further investigation.

The results of these investigations pose many further problems, but clearly the accelerated bone loss in women compared with men is associated with a small fall in dietary intake, a fall in calcium absorption and a higher urinary calcium excretion which fails to fall with age. While dietary deficiency can be a contributory factor to the development of osteoporosis as reported in animals, and seen in patients who have diminished absorption due to gastric surgery or steatorrhoea, the evidence against this being a significant causal factor in the Western urban population is reported in this thesis. The dominant factor is the effect of ageing, which plays a more important part in the development of osteoporosis in women, who show a much greater loss in bone mass with age than is seen in men.

This difference must arise either from a genetic difference affecting the multiplication or function of bone cells, or an age change affecting "la fixite du milieu interieur". The latter could well spring from the changes in hormonal secretion related to the menopause having a direct effect on the bone cells, or on the end effects of diet or hormones on the bone cells.

Certainly, there is no reason to believe that these factors cannot be controlled. Even though the healing of fractures is less efficient in the elderly, there is a considerable increase in the rate of bone turnover at the site of the fracture which indicates that there is no absolute inherent limiting factor within the cell preventing effective cell division and function. However, the nature of these controlling factors have yet to be determined.

S U M M A R Y

SUMMARY

1 The bone mass, as measured by X-ray indices or X-ray densitometry, increases up to between 25 and 40 years of age depending upon the measurement used. All measurements, except cortical density in the male, fall with increasing age thereafter. The cortical density increases throughout life in the male. The X-ray indices, S.A.E. and T.C.M. show an accelerated fall after the menopause in women.

2 The description of investigations in patients who are defined as osteoporotic should be accompanied by measurements which indicate whole bone density, total mineral per unit length of bone, and cortical density. Furthermore, these measurements must be related to the measurements in the normal population of the same age and sex. The best way to quantitate the relation between the patients studied and the normal population is to measure and report the percentile value of the measurement. This has been done in patients with fractures, backache, hyperparathyroidism, hypoparathyroidism, osteomalacia and gastric surgery.

3 Any bone mass measurement which falls with age can be correlated with fracture incidence which rises with age. However, only the total cortical mineral per unit length of bone is related to the rate of change in fracture incidence in both men and women with age, and is the measurement which will correctly define the risk of fracture in both male and female subjects. There is a very marked increase in the incidence rate of fracture after the age of 75 years in both men and women. At this time, the rate of change in bone mass is in fact decreasing. Clearly, this increase in fracture incidence after 75 years of age must be dominated by factors other than the rate of bone mineral loss. Spinal biconcavity and fracture is related to the amount of bone present and not to the percentile value relative to the normal population.

4 Though all the above-mentioned measurements are required to define the bone status, a single measurement can be used to describe a patient as being osteoporotic, but this measurement must take into account the size of the individual. A convenient measurement which fulfills this criterion is the whole bone density of the 3rd metacarpal (S.A.E.). The value of the S.A.E. which gives a useful dividing line is 28. There is a considerably higher incidence of spinal biconcavity and fracture of the femoral neck in women when the whole bone density falls below this value. This value also corresponds with the 5 percentile value at the age of 35 years, when the whole bone density is greatest for both men and women.

5 All subjects, but especially women, lose bone with age. In the absence of any predisposing factors such as gastric surgery or steatorrhoea there is no evidence that there is a specific group of patients in whom the rate of bone loss is greater than in the normal population. The mean bone mass in patients with symptoms, such as backache, or fracture, do not differ significantly from the normal population. Furthermore, while osteoporosis is a major problem in the elderly woman, it is of much less significance in the male.

6 The rate of bone mineral transfer in women falls with age. The urinary hydroxyproline excretion, which correlates with the rate of bone destruction, again measured with isotopes, rises with age. Though the bone mineral transfer rate does not measure the true bone formation rate quantitatively, the experimental evidence in dogs quoted in Chapter VI is that the bone mineral transfer rate correlates with the rate of bone formation.

The evidence therefore is in keeping with a fall in the true bone formation rate with age and a rise in the bone destruction rate in women. However, as the bone mass falls with age, the rate of bone formation per unit mass does not change significantly. There is, however, a significant rise in the rate of bone resorption with age.

7 These studies show that the bone loss with age in women is associated with decreasing dietary intake, diminishing calcium absorption, a failure to attain a corresponding fall in urinary calcium excretion, a fall in the rate of bone formation and a rise in the rate of bone destruction with age. Normal male subjects also show a slight fall in dietary intake with age. They do not, however, show any fall in calcium absorption. The calcium absorption in male subjects remains significantly better than in the females at each decade between the 4th and 7th. The male subjects also showed no significant fall in urinary calcium excretion with age, but their urinary calcium:creatinine ratios were consistently lower than that of the female subjects, though these differences did not reach statistical significance at except in the 4th and 5th decades.

These differences between the male and female subjects account for the differences in the rate of loss of bone between the sexes. However, these observed effects could be the cause or the result of the decreasing bone mass with age.

8 The effects of calcium supplementation of the diet on bone mass in 94 subjects was studied from 18 to 54 months. Four patients showed a significant increase in the bone mass over the period of the study. Twenty-three patients showed a significant fall in bone mass. The remainder showed no significant change. (There were no features which clearly distinguished any group from the normal subjects or the remaining patients. When considered as a group, the fall in bone mass over the period of study was significantly greater than that observed in the normal population of a corresponding age. The evidence implicates supplementary calcium as the cause of the accelerated bone loss.

9 The evidence in 8 above is at variance with the reported positive balances in patients given calcium supplements, and with the evidence in Chapter VI of a fall in bone destruction rate which correlated inversely with the calcium balance, and the fall in urinary hydroxyproline which correlated with the fall in bone destruction rate. Clearly, either the balance results have a consistent error, or else the benefit from calcium supplements is only temporary. There is an increase in faecal bile acids and faecal fat in patients on calcium supplements. Malabsorption produced by the calcium supplements may therefore eventually cause the calcium balance to again become negative.

10 The small fall in vitamin D intake with age, the accelerated fall in bone mass in steatorrhoea and following gastric surgery, the fall in calcium absorption with age, the reported lower values of serum anti-rachitic activity in osteoporosis (Smith et al, 1964), and the increased faecal fat output in patients on calcium supplements, and increased rate of loss of bone when compared with normal subjects suggest that the role of vitamin D in the development of primary and secondary osteoporosis requires further investigation.

11 While dietary deficiency can be a contributory factor to the development of osteoporosis in experimental animals, there is no evidence that it contributes to the development of osteoporosis in the Western urban population. The dominant factor is the effect of ageing which affects women more than men. This difference must arise from the genetic difference between the sexes. The proximal cause must be either a change in hormonal secretion at the menopause affecting the relative rates of bone formation and destruction, or a genetically determined failure of cell multiplication or function.

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DATE OF BIRTH: 12/4/27 **DATE OF GRADUATION AS M.B.,Ch.B. (GLASGOW)** 1957

OTHER QUALIFICATIONS	AWARDING INSTITUTION	DATE OF AWARD
B.Sc.	Univ. of Glasgow	1952
M.R.C.P.	Royal Coll. of Physicians of Edinburgh	1961

MEDICAL APPOINTMENTS HELD SINCE GRADUATION

Designation of Post	where held	from	to
Research Asst.(M.R.C.)	Univ.Dept.Med. W.I.G.	1961	1964
Lecturer (Hon. Senior Registrar)	Univ.Dept.Med. W.I.G.	1964	1970

NAME AND ADDRESS OF GENERAL PRACTICE, HOSPITAL, DEPARTMENT, LABORATORY OR OTHER INSTITUTION WHERE WORK FOR THIS THESIS WAS UNDERTAKEN

University Department of Medicine, Gardiner Institute,
Western Infirmary, Glasgow, W.1.

DECLARATION I declare that the work has been done and the thesis composed by myself, and that the books and papers cited were all consulted by me personally, unless it is otherwise stated.

(NOTE: where material based on work undertaken in collaboration with others is included in the thesis a further and separate statement must be submitted clearly defining the candidate's individual contribution)

DATE: 8/9/70 **SIGNED:** D.A. Stuart-Smith

CERTIFICATION:

I hereby certify that the above named candidate for the degree of M.D. has been engaged since graduation for at least one year either in scientific work bearing directly on his profession or in the practice of Medicine.

PERIOD CERTIFIED: 1961 - 1970 **SIGNED:** G M Wilson

DATE: 8th September, 1970. **ADDRESS:** University Dept. of Medicine, Western Infirmary, Glasgow, W.1.

POSITION: Regius Professor of the Practice of Medicine.

mm
15/9/70

UNIVERSITY OF GLASGOW

DEGREE OF M.D.

FORM OF APPLICATION

NOTES

1. Before completing the form on the facing page, please read carefully the supplementary regulations below.

2. If at all possible, the form should be typewritten.

3. The completed form should be returned to the Clerk of the Faculty of Medicine, the University, Glasgow, W.2., with the fee of £30 and two copies of the thesis by .....

REGULATIONS

Extract from Ordinance for Degree of Doctor of Medicine

"(1) A candidate for the Degree of Doctor of Medicine, or for the degree of Master of Surgery, shall be a graduate of the University in Medicine and Surgery of at least two years' standing and shall have been engaged since graduation for at least one year either in scientific work bearing directly on his profession, or in the practice of Medicine or Surgery, respectively.

(2) A candidate for the Degree of Doctor of Medicine, or for the Degree of Master of Surgery, may be required to pass an examination in such a department or departments of medical or surgical science or practice as the Senatus may prescribe or approve.

(3) A candidate for the Degree of Doctor of Medicine, or for the Degree of Master of Surgery, shall submit for the approval of the Faculty of Medicine a thesis on any branch of knowledge comprised in or related to the curriculum for the Degrees of Bachelor of Medicine and Bachelor of Surgery, and may be required to present himself for oral or other examination in the subject-matter thereof: provided that a candidate for the Degree of Doctor of Medicine shall not submit a thesis on a subject which is exclusively surgical, and that a candidate for the Degree of Master of Surgery shall not submit a thesis on a subject which is exclusively medical.

(4) The thesis shall be presented at such time and in accordance with such regulations as the Senatus may prescribe.

(5) The Examiners for the Degrees shall be the Professors, Readers and Lecturers in the Faculty of Medicine together with such other internal and and additional Examiners as the Court shall appoint.

SUPPLEMENTARY REGULATIONS

1. Two copies of each thesis are required. Theses must be type-written on paper approximately 10 inches by 8 inches and bound in dark cloth with stiff boards. The title of each thesis and the name of the author must be printed in block letters on the outside binding. Theses should be lodged with the Dean of the Faculty of Medicine not later than 15th September, or 15th December, or 15th March, for adjudication during the Martinmas, Candlemas, and Whitsun terms respectively.

2. A thesis will not be approved unless it gives evidence of original observation, or, if it deals with the researches of others, gives a full statement of the literature of its subject with accurate references and critical investigation of the views or facts cited: mere compilations will in no case be accepted.

3. A thesis must be a dissertation written for the purpose, provided that the results of original observations already published in medical or scientific journals or in the transactions of learned societies or otherwise may be accepted in place of such a dissertation. Published papers submitted in lieu of a dissertation must be related and accompanied by a statement, preferably in the form of an introductory paper, showing the relationship between the various studies and placing the whole work critically into perspective with the general state of knowledge in the field of investigation to which the candidate's researches are related.

4. A declaration signed by the candidate that the work has been done and the thesis composed by himself must be submitted with the thesis. Where material based on work undertaken in collaboration with others is included in the thesis a separate statement clearly defining the extent of his personal contribution must also be submitted by the candidate. If the whole or any part of the subject matter of the thesis has been included in a thesis already approved for a degree in this or another University, the candidate must make a declaration to this effect and must lodge with his thesis either a copy of such previously approved thesis or a precise statement of its scope.

5. Two copies of a separate summary (500-1000 words) giving an adequate and informative abstract of the work, must be submitted with the thesis.

6. A thesis approved for the Degree of Doctor of Medicine or Master of Surgery may be deemed to be (i) Worthy of Honours or (ii) Worthy of Commendation or (iii) Sufficient.

7. If the thesis is approved, the copies submitted by the candidate shall become the property of the University."

MEDICAL APPOINTMENTS HELD SINCE GRADUATION

Designation of Post	where held	from	to
House Physician	Professorial Medical Unit, Glasgow Royal Infirmary	1957	1958
House Surgeon	Professorial Surgical Unit, Glasgow Royal Infirmary	1958	1958
Ure Research Scholarship	University Dept. Pathology, Glasgow Royal Infirmary	1958	1959
Senior House Officer	Department of Haematology, Glasgow Royal Infirmary	1959	1960
Senior House Officer	with Dr. J.H. Wright, Glasgow Royal Infirmary	1960	1961
Research Assistant (M.R.C.)	University Dept. Medicine, Gardiner Institute, Western Infirmary, Glasgow.	1961	1964

PRESENT APPOINTMENT

Lecturer in Medicine		University Dept. Medicine, Gardiner Institute, Western Infirmary, Glasgow.	1964
Hon. Senior Registrar			1965
	now at	Bone Metabolism Research Unit, Western Infirmary, Glasgow, W.1.	

O S T E O P O R O S I S

THESIS PRESENTED TO THE UNIVERSITY OF GLASGOW FOR THE DEGREE OF M.D.

S U M M A R Y

SEPTEMBER, 1970.

Deryk A. Stuart-Smith, Esq., B.Sc., M.B., Ch.B., M.R.C.P.(E),
Bone Mineral Metabolism Research Unit,
Western Infirmary,
G L A S G O W.

1 The bone mass, as measured by X-ray indices or X-ray densitometry, increases up to between 25 and 40 years of age depending upon the measurement used. All measurements, except cortical density in the male, fall with increasing age thereafter. The cortical density increases throughout life in the male. The X-ray indices, whole bone density (S.A.E.) and the total cortical mineral per unit length of bone (T.C.M.) show an accelerated fall after the menopause in women.

2 The description of investigations in patients who are defined as osteoporotic should be accompanied by measurements which indicate whole bone density, total mineral per unit length of bone and cortical density. Furthermore, these measurements must be related to the measurements in the normal population of the same age and sex. The best way to quantitate the relation between the patients studied and the normal population is to measure and report the percentile value of the measurement. This has been done in patients with fractures, backache, hyperparathyroidism, hypoparathyroidism, osteomalacia and gastric surgery.

Any bone mass measurement which falls with age can be correlated with fracture incidence which rises with age. However, only the total cortical mineral per unit length of bone is related to the rate of change in fracture incidence in both men and women with age, and is the measurement which will correctly define the risk of fracture in both male and female subjects. There is a very marked increase in the incidence rate of fracture after the age of 75 years in both men and women. At this time, the rate of change in bone mass is in fact decreasing. Clearly, this increase in fracture incidence after 75 years of age must be dominated by factors other than the rate of bone mineral loss. Spinal biconcavity and fracture is related to the amount of bone present and not to be percentile value relative to the normal population.

Though all the above-mentioned measurements are required to define the bone status, a single measurement can be used to describe a patient as being osteoporotic, but this measurement must take into account the size of the individual. A convenient measurement which fulfills this criterion is the whole bone density of the 3rd metacarpal (S.A.E.). The value of the S.A.E. which gives a useful dividing line is 28. There is a considerably higher incidence of spinal biconcavity and fracture of the femoral neck in women when the whole bone density falls below this value. This value also corresponds with the 5 percentile value at the age of 35 years, when the whole bone density is greatest for both men and women.

5 All subjects, especially women, lose bone with age. In the absence of any predisposing factors such as gastric surgery or steatorrhoea there is no evidence that there is a specific group of patients in whom the rate of bone loss is greater than in the normal population. The mean bone mass in patients with symptoms, such as backache, or fracture, do not differ significantly from the normal population. Furthermore, while osteoporosis is a major problem in the elderly woman, it is of much less significance in the male.

6 The rate of bone mineral transfer in women falls with age. The urinary hydroxyproline excretion, which correlates with the rate of bone destruction, again measured with isotopes, rises with age. Though the bone mineral transfer rate does not measure the true bone formation rate quantitatively, the experimental evidence in dogs quoted in Chapter VI is that the bone mineral transfer rate correlates with the rate of bone formation.

The evidence therefore is in keeping with a fall in the true bone formation rate with age and a rise in the bone destruction rate in women. However, as the bone mass falls with age, the rate of bone formation per unit mass does not change significantly. There is, however, a significant rise in the rate of bone resorption with age.

7 The dietary intake of calcium, phosphorus, protein and vitamin D falls significantly with age in women. There was a small, but not significant, fall in dietary intake with age in men. Men have a significantly higher intake of calcium, phosphorus, protein and vitamin D than women. These differences, however, were no longer apparent when age and weight were taken into consideration. The dietary intake of calcium, phosphorus, protein and vitamin D at each decade between the 4th and the 7th was examined in women with X-ray indices above and below the mean in each group.

Only between 51 and 60 years were small differences between the dietary intakes found, and these were related only to the Relative Vertebral Density (R.V.D.). The evidence therefore shows that some factor connected with age and not diet is responsible for the loss of bone in women.

8 These studies show further that the bone loss with age in women is significantly associated with decreasing dietary intake, diminishing calcium absorption, a failure to attain a corresponding fall in urinary calcium excretion, a fall in the rate of bone formation and a rise in the rate of bone destruction with age. Normal male subjects also show a slight fall in dietary intake with age. They do not, however, show any fall in calcium absorption. The calcium absorption in male subjects remains significantly better than in the females at each decade between the 4th and 7th. The male subjects also showed no significant fall in urinary calcium excretion with age, but their urinary calcium:creatinine ratios were consistently lower

than that of the female subjects, though these differences did not reach statistical significance except in the 4th and 5th decades.

These differences between the male and female subjects account for the differences in the rate of loss of bone between the sexes. However, these observed effects could be the cause or the result of the decreasing bone mass with age.

9 The effects of calcium supplementation of the diet on bone mass in 94 subjects was studied from 18 to 54 months. Four patients showed a significant increase in the bone mass over the period of the study. Twenty-three patients showed a significant fall in bone mass. The remainder showed no significant change. There were no features which clearly distinguished any group from the normal subjects or the remaining patients. When considered as a group, the fall in bone mass over the period of study was significantly greater than that observed in the normal population of a corresponding age. The evidence implicates supplementary calcium as the cause of the accelerated bone loss.

10 The evidence in 9 above is at variance with the reported positive balances in patients given calcium supplements, and with the evidence in Chapter VI of a fall in bone destruction rate which correlated inversely with the calcium balance, and the fall in urinary hydroxyproline which correlated with the fall in bone destruction rate.
Clearly, either the balance results have a consistent error, or else the benefit from calcium supplements is only temporary.
This was examined further and a significant increase in faecal bile acids and faecal fat in patients on calcium supplements was found.
Malabsorption produced by the calcium supplements may therefore eventually cause the calcium balance to again become negative.

11 The small fall in vitamin D intake with age, the accelerated fall in bone mass in steatorrhoea and following gastric surgery, the fall in calcium absorption with age, the reported lower values of serum anti-rachitic activity in osteoporosis (Smith et al, 1964), and the increased faecal fat output in patients on calcium supplements, and increased rate of loss of bone when compared with normal subjects, suggest that the role of vitamin D in the development of primary and secondary osteoporosis requires further investigation.

12 While dietary deficiency can be a contributory factor to the development of osteoporosis in experimental animals, there is no evidence that it contributes to the development of osteoporosis in the Western urban population. The dominant factor is the effect of ageing which affects women more than men. This difference must arise from the genetic difference between the sexes. The proximal cause must be either a change in hormonal secretion at the menopause affecting the relative rates of bone formation and destruction, or a genetically determined failure of cell multiplication or function.

O S T E O P O R O S I S

THESIS PRESENTED TO THE UNIVERSITY OF GLASGOW FOR THE DEGREE OF M.D.

SEPTEMBER, 1970

Deryk A. Stuart-Smith, Esq., B.Sc., M.B., Ch.B., M.R.C.P.(E),
Bone Mineral Metabolism Research Unit,
Western Infirmary,
G L A S G O W.

TABLE OF CONTENTS

Volume II

CHAPTER I

TABLE I

Distribution of subjects by their Socio-economic class. In the case of the female subjects the social class recorded is that of their husband if married, or their own if widowed or single (Appendix B1 of the Registrar General's Classification of Occupations (1960). The last two columns show the percentage of subjects in each socio-economic group in the Central Clydeside Conurbation (1966 Census).

CLASS	Female subjects (Group I)		Male subjects (Group II)		Female subjects (Group III)		Male subjects (Group IV)		Registrar General's figures	
	Number	%age	Number	%age	Number	%age	Number	%age	Male	Female
1) Professional etc.	6	4.3	1	1.3	12	3.8	20	6.4	3.3	0.6
2) Senior Executive	15	9.8	11	14.7	39	12.3	48	15.4	10.4	13.1
3) Skilled	100	68.1	33	44.0	139	43.8	140	44.9	53.7	49.5
4) Semi-skilled	18	12.8	18	23.9	72	22.7	68	21.8	18.7	27.0
5) Unskilled	7	5.7	6	8.0	28	8.8	22	7.1	12.5	9.2
Not recorded	6	4.3	6	8.0	27	8.4	14	4.5	1.4	0.6
TOTAL	152		75		317		312			

TABLE II

Analysis of medical histories in Group I (152 normal female subjects), Group II (75 normal male subjects), Group III (317 normal female subjects) and Group IV (312 normal male subjects).

History	Group I	Group II	Group III	Group IV
Fracture	23	15	54	43
Renal stone	4	3	4	13
Persistent nocturia	42	13	58	44
Frequency of micturition	16	4	24	25
Dysuria	14	1	14	2
Diarrhoea (for over 6 months)	5	2	1	1
Chronic bronchitis	10	6	8	24
Gastroenterostomy	1	0	2	4

TABLE III

Regression equations of Metacarpal Index, Femoral Index, Spinal Index and Relative Vertebral Density on age in Group I (152 normal female volunteers) and Group II (75 normal male volunteers).

	Regression equation $\pm$ 2 S.E.	Correlation coefficient (r)	P
Female subjects			
Metacarpal index	$y = 0.82 - 0.005x \pm 0.15$	-0.64	< 0.001
Femoral index	$y = 0.68 - 0.002x \pm 0.13$	-0.40	< 0.001
Spinal index	$y = 0.94 - 0.001x \pm 0.10$	-0.26	< 0.005
Relative Vertebral Density	$y = 3.53 - 0.06\ x \pm 2.5$	-0.50	< 0.001
Male subjects			
Metacarpal index	not significant		
Femoral index	$y = 0.065 - 0.0017x \pm 0.17$	-0.32	< 0.01
Spine index	not significant		
Relative Vertebral Density	not significant		

TABLE IV

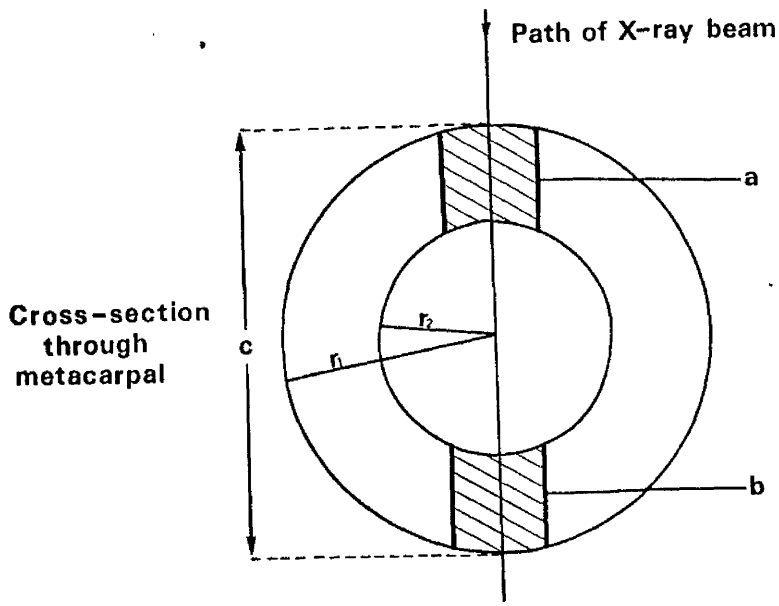
Comparison between normal male and female subjects of Standardised Aluminium Equivalent (S.A.E.) and density (λ) of the 3rd metacarpal. Differences between means are considered not to be significant (N.S.) unless P < 0.05.

Age group	S.A.E. Mean Female subjects	S.A.E. Mean Male subjects	S.A.E. 't'	S.A.E. P	λ Mean Female subjects	λ Mean Male subjects	λ 't'	λ P
5 – 9	20.9	21.1	0.15	N.S.	4.41	4.78	1.29	N.S.
10 – 14	27.9	22.3	1.73	N.S.	5.73	4.61	2.83	N.S.
15 – 19	33.3	31.9	0.73	N.S.	5.40	5.25	0.65	N.S.
20 – 24	34.1	32.7	0.92	N.S.	5.55	5.32	1.27	N.S.
25 – 29	36.5	33.9	1.87	N.S.	5.74	5.59	1.05	N.S.
30 – 34	35.4	35.5	0.07	N.S.	5.80	5.45	1.93	N.S.
35 – 39	35.7	34.6	0.60	N.S.	5.95	5.47	2.52	N.S.
40 – 44	35.1	32.1	1.99	N.S.	5.72	5.50	1.53	N.S.
45 – 49	34.7	33.8	0.61	N.S.	5.78	5.56	1.34	N.S.
50 – 54	32.9	33.7	0.58	N.S.	5.73	5.79	0.38	N.S.
55 – 59	29.2	31.5	1.82	N.S.	5.74	5.53	1.31	N.S.
60 – 64	28.5	29.9	1.48	N.S.	5.75	5.65	0.58	N.S.
65 – 69	26.3	31.0	4.21	< .001	5.65	5.71	0.33	N.S.
70 – 74	24.3	29.7	4.58	< .001	5.70	5.72	0.12	N.S.
75 – 79	23.5	30.8	3.30	< .001	5.54	6.38	2.39	< 0.05
80 +	21.8	25.8	1.14	N.S.	5.58	6.37	0.72	N.S.

TABLE V

Comparison of S.A.E. in male and female subjects in social classes 1 + 2 and 4 + 5 in relation to age.

Age group	Social class	Mean S.A.E. Female	Mean S.A.E. Male	P Female	P Male
20 - 29	1 + 2	35.57	32.68	N.S.	N.S.
	4 + 5	34.85	34.27		
30 - 39	1 + 2	32.39	34.11	N.S.	N.S.
	4 + 5	35.74	36.29		
40 - 49	1 + 2	36.94	32.37	N.S.	N.S.
	4 + 5	33.87	31.72		
50 - 59	1 + 2	33.57	30.23	N.S.	N.S.
	4 + 5	30.54	33.84		
60 - 69	1 + 2	26.63	33.99	N.S.	< .01
	4 + 5	28.57	29.67		
70 +	1 + 2	24.48	28.72	N.S.	N.S.
	4 + 5	24.36	29.05		



A.E. = Absorption equivalent in Al of (a+b)

$$S.A.E. = \frac{A.E.}{c}$$

λ = Linear attenuation coefficient of the X-radiation in (a+b)

$$T.C.M. = \lambda \times \pi (r_1^2 - r_2^2)$$

$$S.T.C.M. = \frac{T.C.M.}{r_1^2}$$

Fig 1 The estimation of aluminium equivalent (A.E.) and the calculation of the Standardised Aluminium Equivalent (S.A.E.), Lambda (λ), Total Cortical Mineral per Unit Length of bone (T.C.M.) and Standardised Total Cortical Mineral per unit length (S.T.C.M.) of the right 3rd metacarpal.

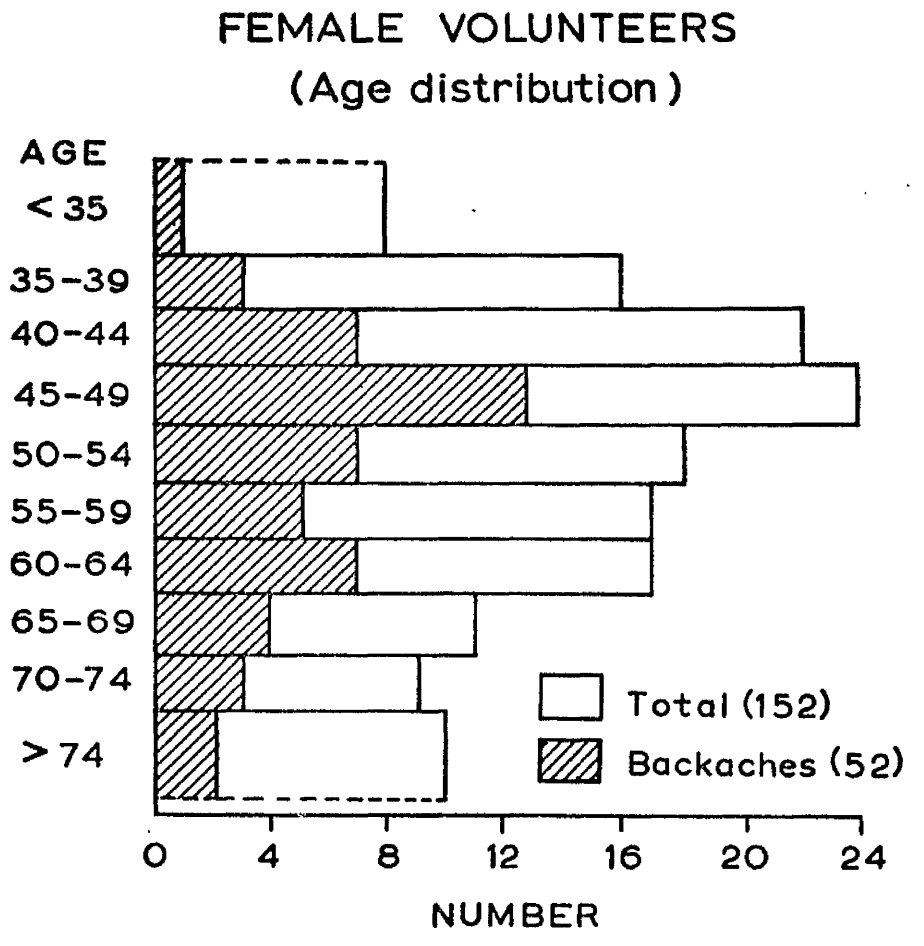


Fig 2 The distribution of normal female subjects (Group I) in 5 year age groups, showing the incidence of backache in each age group (152 subjects).

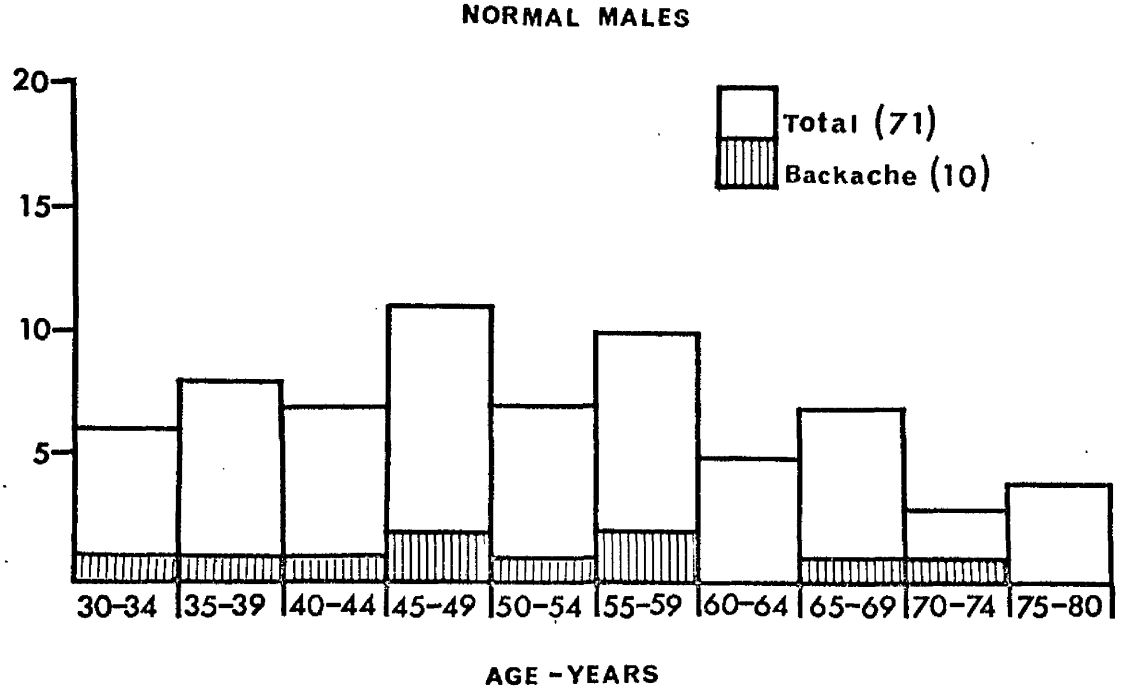


Fig 3 The distribution of normal male subjects (Group II) in 5 year age groups, showing the incidence of backache in each age group (75 subjects).

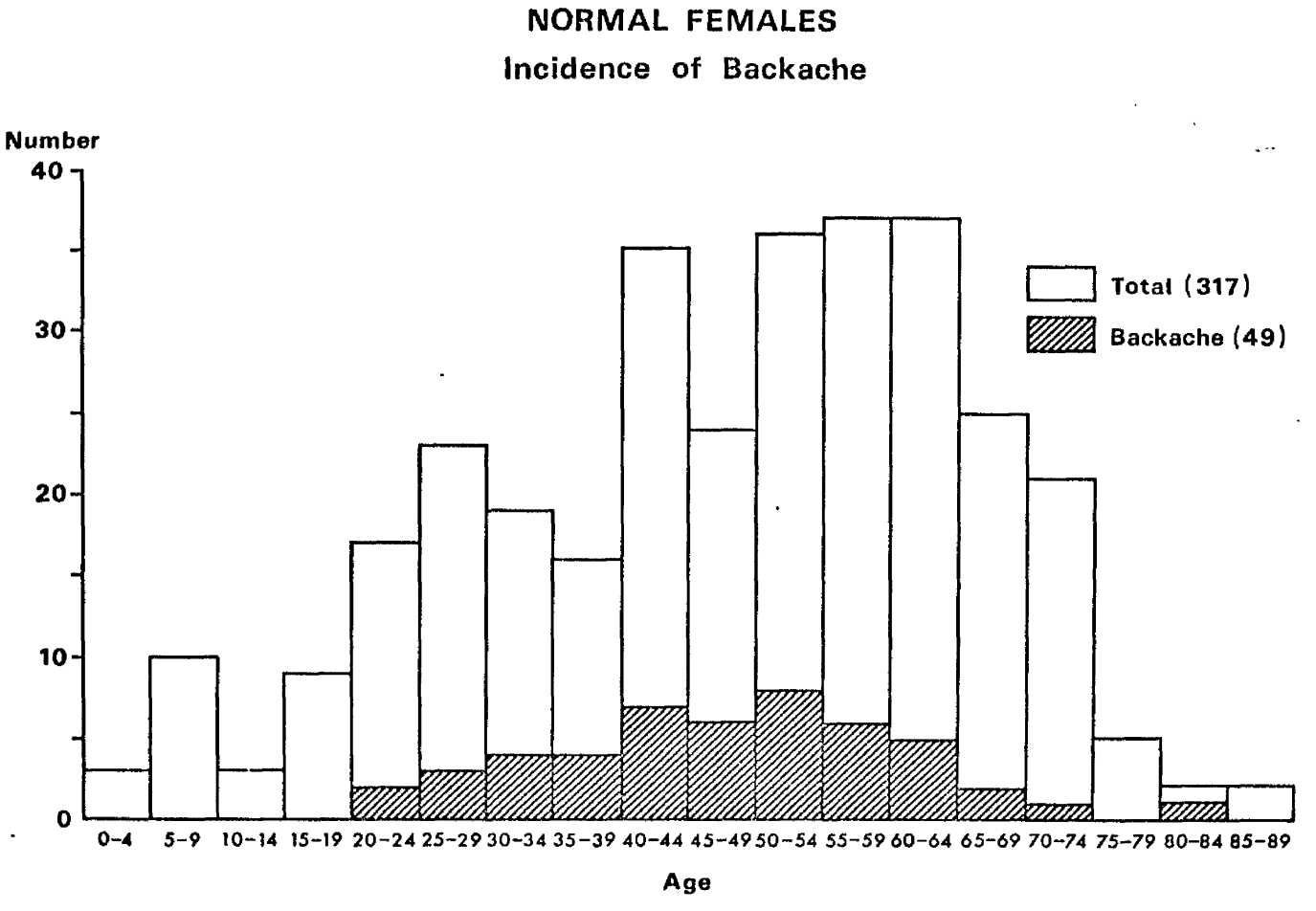


Fig 4 The distribution of normal female subjects (Group III) in 5 year age groups, showing the incidence of backache in each age group (317 subjects).

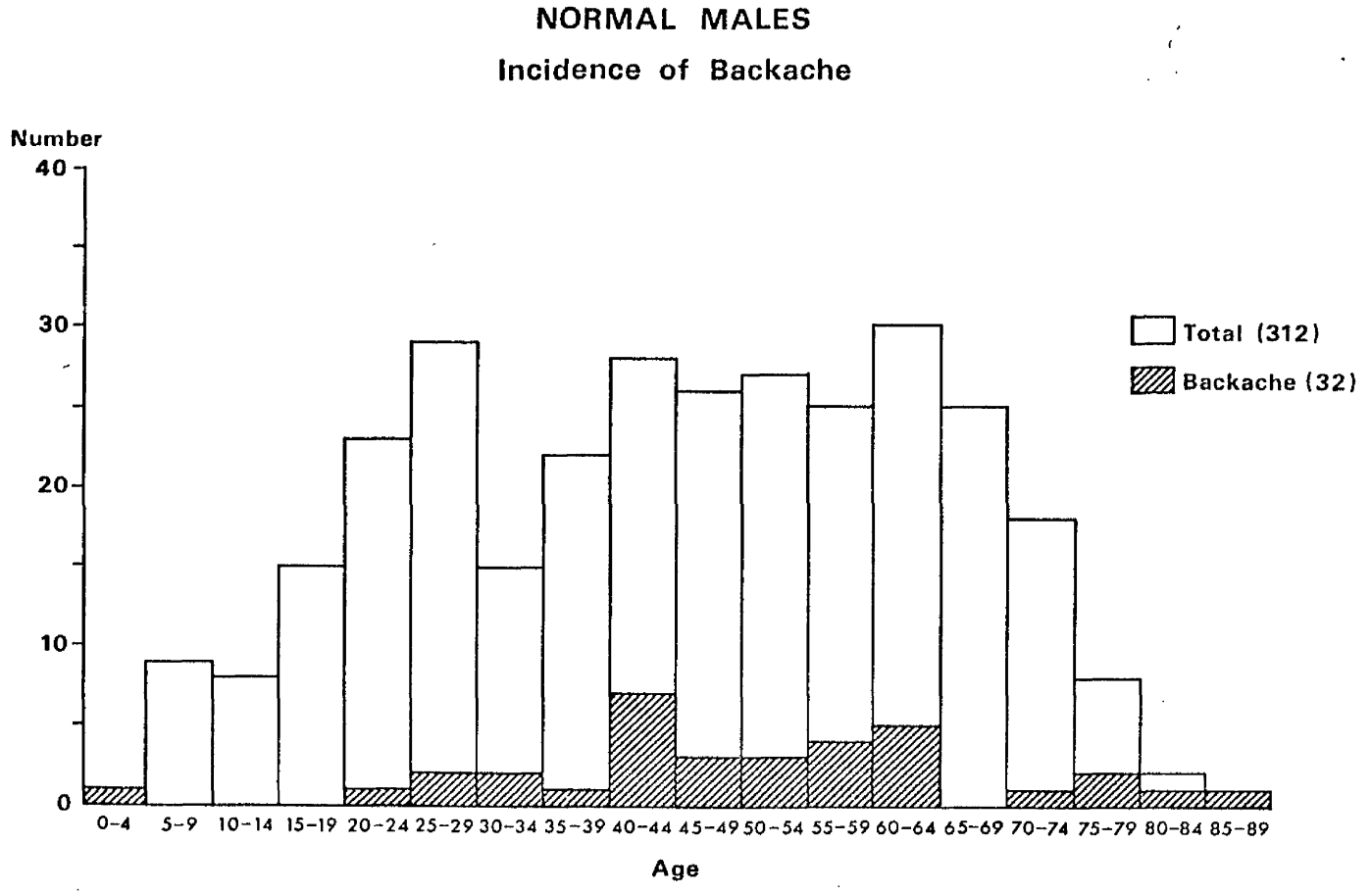


Fig 5 The distribution of normal male subjects (Group IV) in 5 year age groups, showing the incidence of backache in each age group (312 subjects).

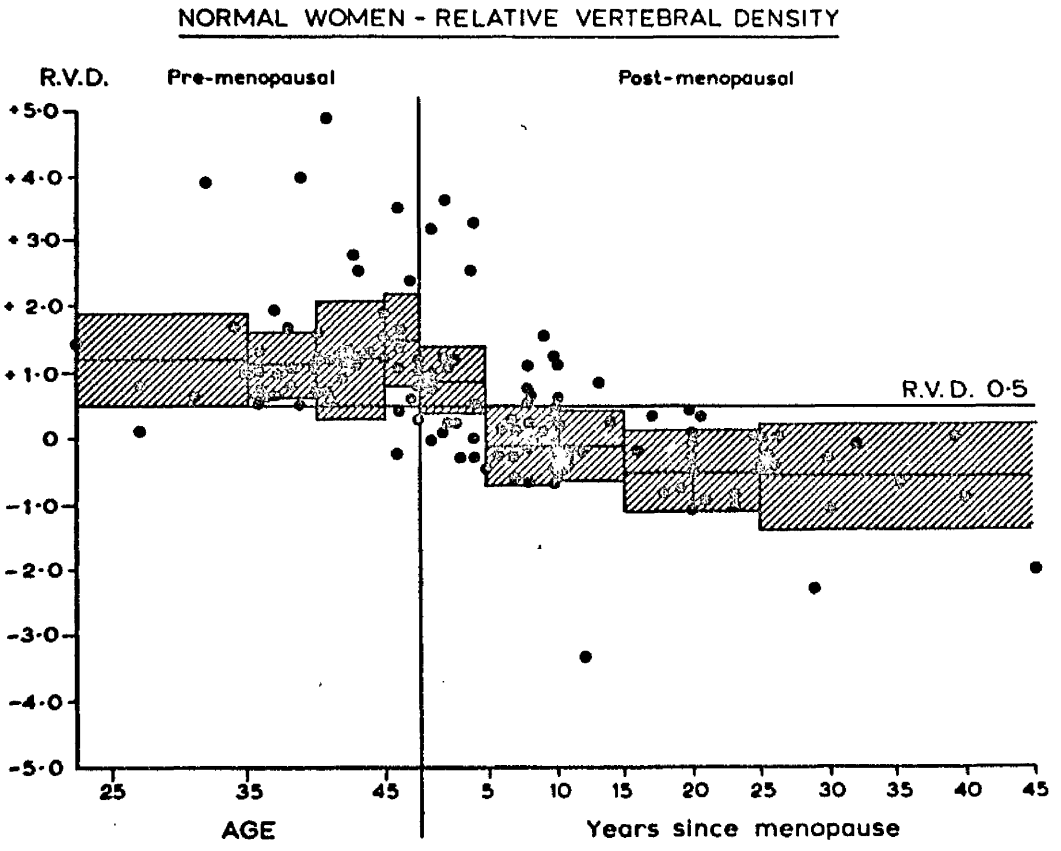


Fig 6 The relation between Relative Vertebral Density (R.V.D.) and the menopause in 152 normal female subjects (Group I). The R.V.D. and the two S.E. range for each 5 year age group is shown. The age of the subjects is shown in the pre-menopausal volunteer and the time since the onset of the menopause in those who have past the menopause. There is an abrupt fall in density 5 years after the onset of the menopause.

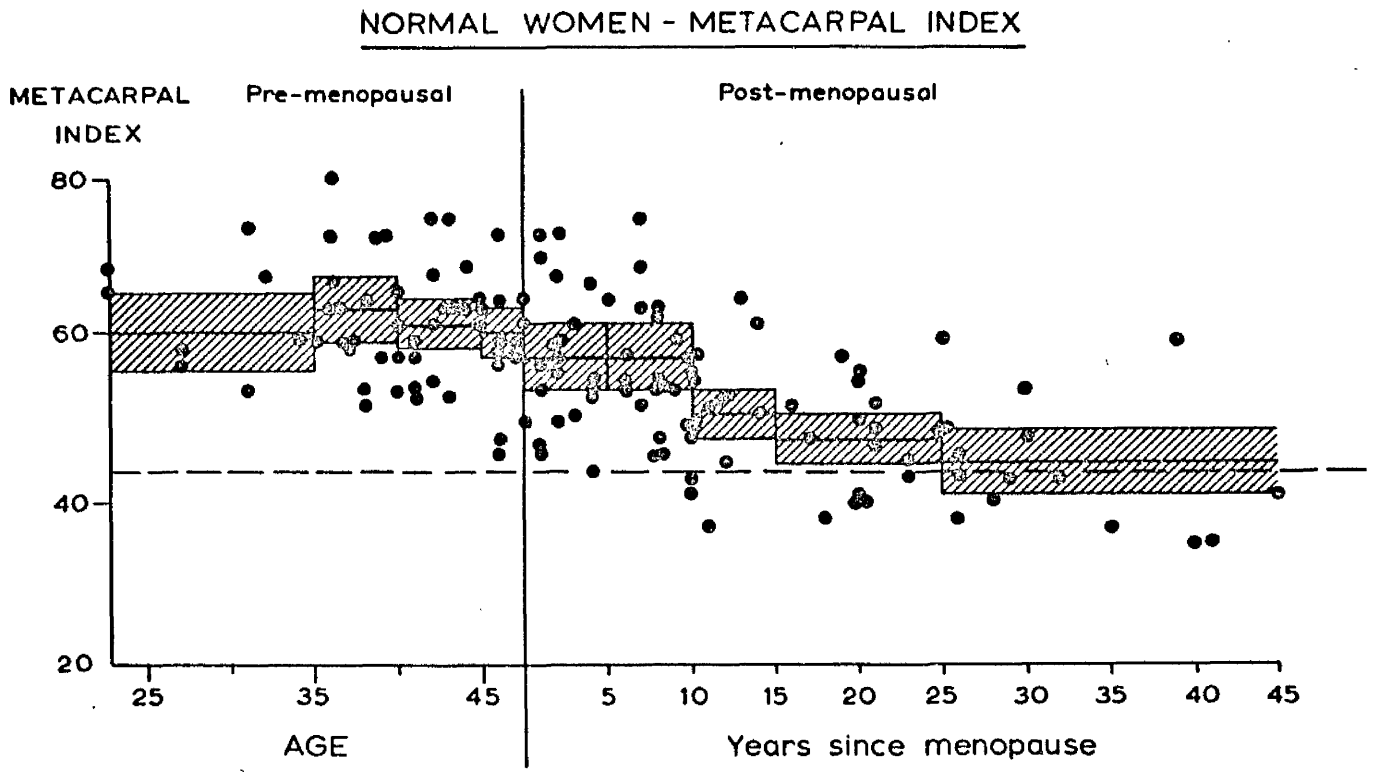


Fig 7 The relation between the Metacarpal Index (M.I.) and the menopause in 152 normal females (Group I). The mean and two S.E. range for each 5 year age group is shown. The age of the volunteers is shown in the pre-menopausal subjects and the time since the onset of the menopause in those who have past the menopause. There is an abrupt fall in the M.I. 10 years after the onset of the menopause.

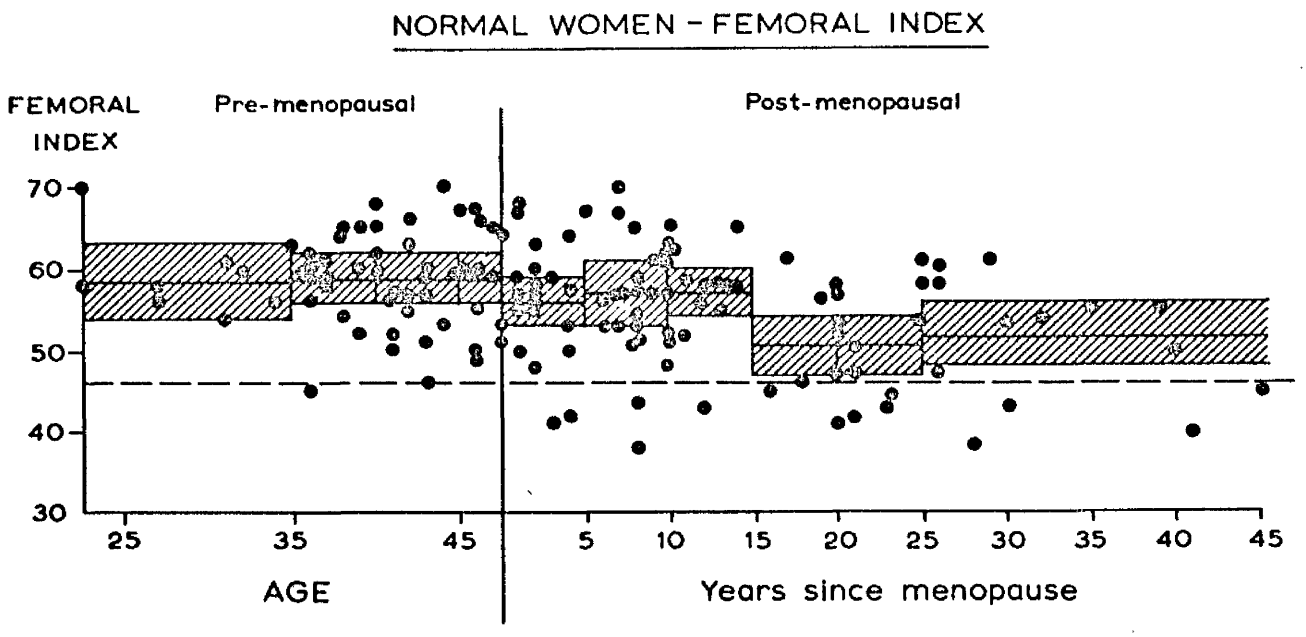


Fig 8 The relation between the Femoral Index (F.I.) and the menopause in 152 normal female subjects (Group I). The F.I. of each subject and the two S.E. range for each 5 year age group is shown. The age of the volunteers are shown in the pre-menopausal subjects, and the time since the onset of the menopause in those who are past the menopause. There is an abrupt fall in the F.I. 15 years after the onset of the menopause.

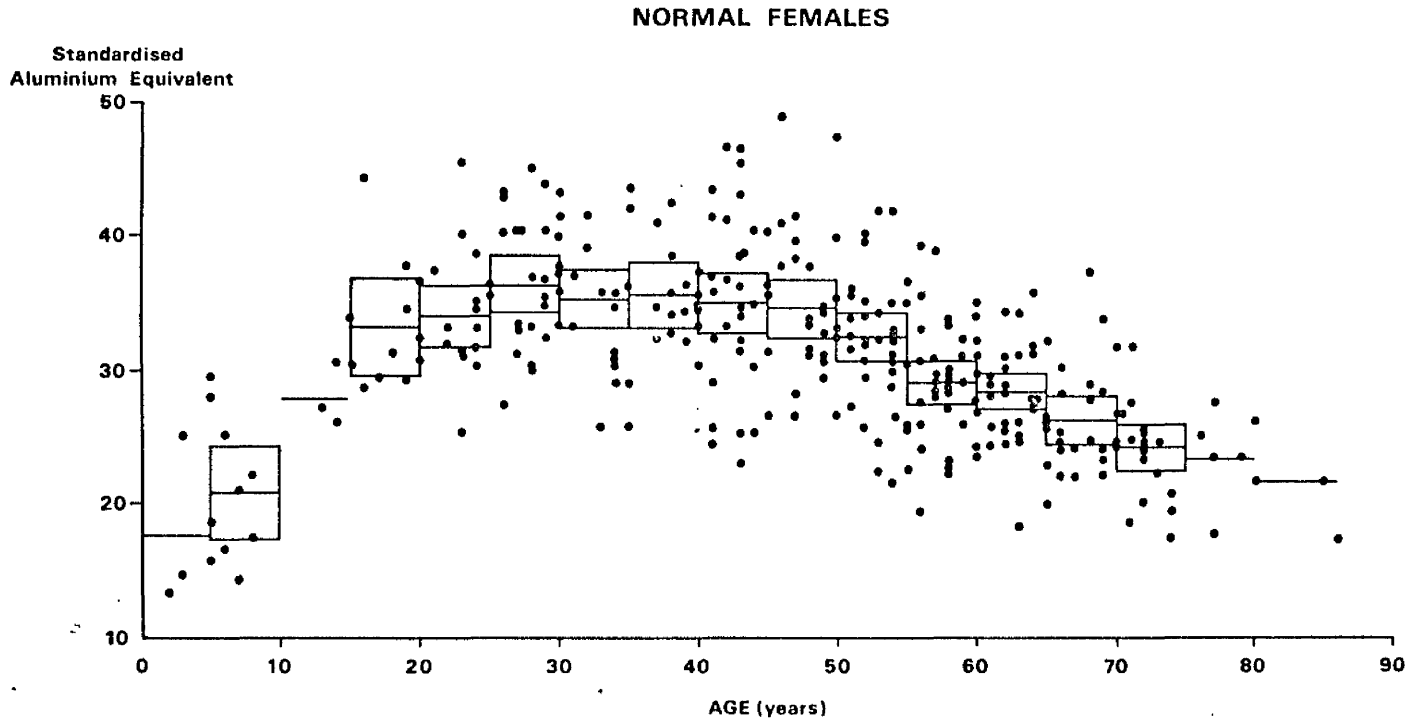


Fig 9 The change in whole bone density (S.A.E.) of the 3rd metacarpal with age in 317 normal female subjects (Group III). The mean and two S.E. range for each 5 year age group is shown.

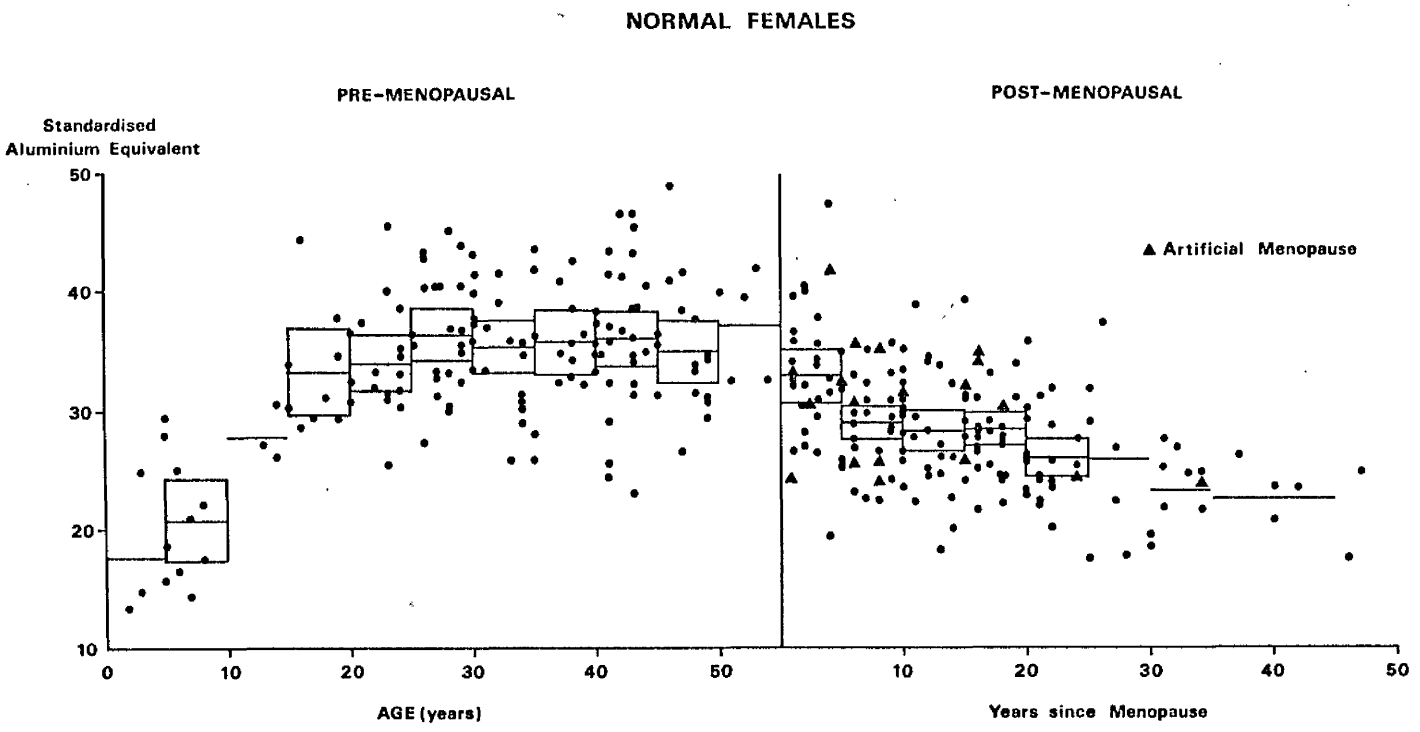


Fig 10 The relation between the whole bone density (S.A.E.) and the menopause in 317 normal female subjects (Group III) The S.A.E. of each subject and the 2 S.E. range for each 5 year age group is shown. The age of the volunteer is shown in the pre-menopausal subjects and the time since the onset of the menopause in those subjects who have past the menopause. There is an abrupt fall in S.A.E. 5 years after the onset of the menopause.

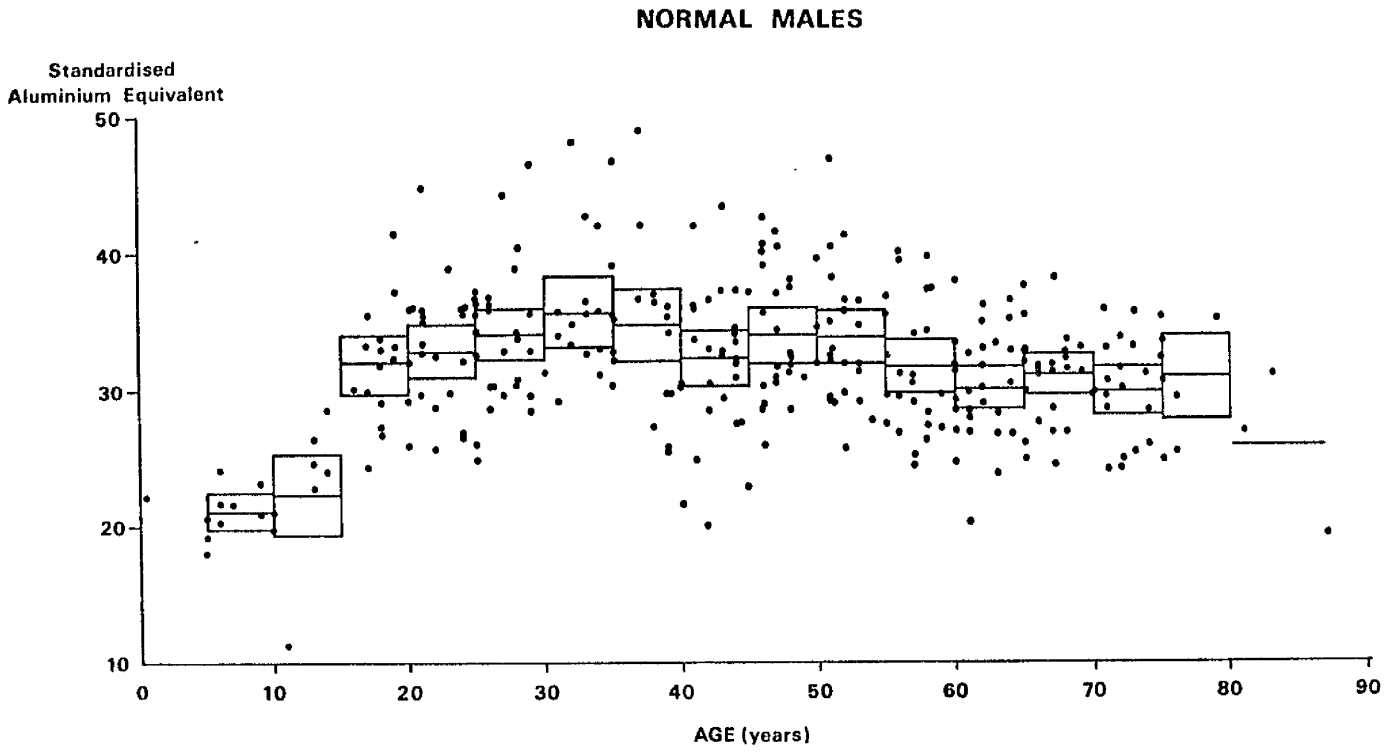


Fig 11 The relation between whole bone density (S.A.E.) and age in 312 normal male subjects (Group IV). The S.A.E. for each individual and the mean and two S.E. range for each 5 year group is indicated.

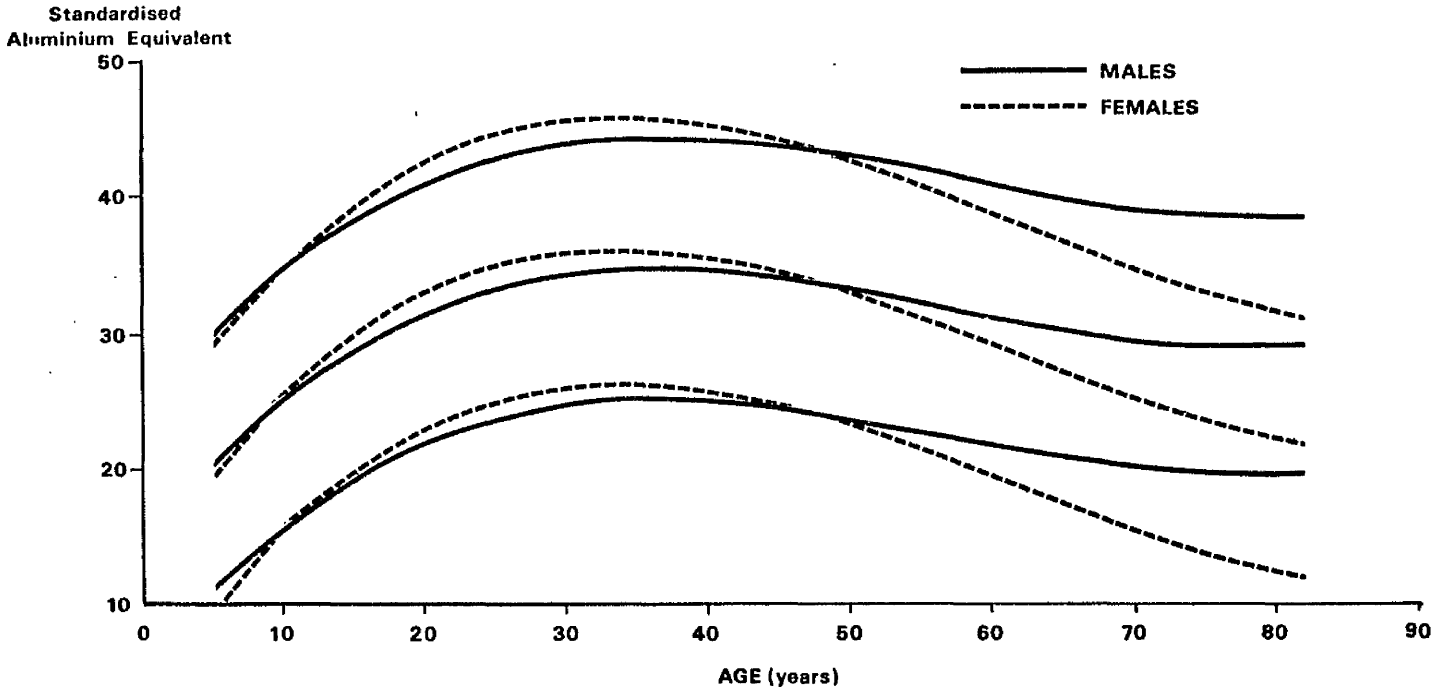


Fig 12 The change in whole bone density (S.A.E.) with age comparing normal male and female subjects (Groups III and IV). The data has been analysed in terms of a third order polynomial. The mean and the 5 to 95 percent limits are shown.

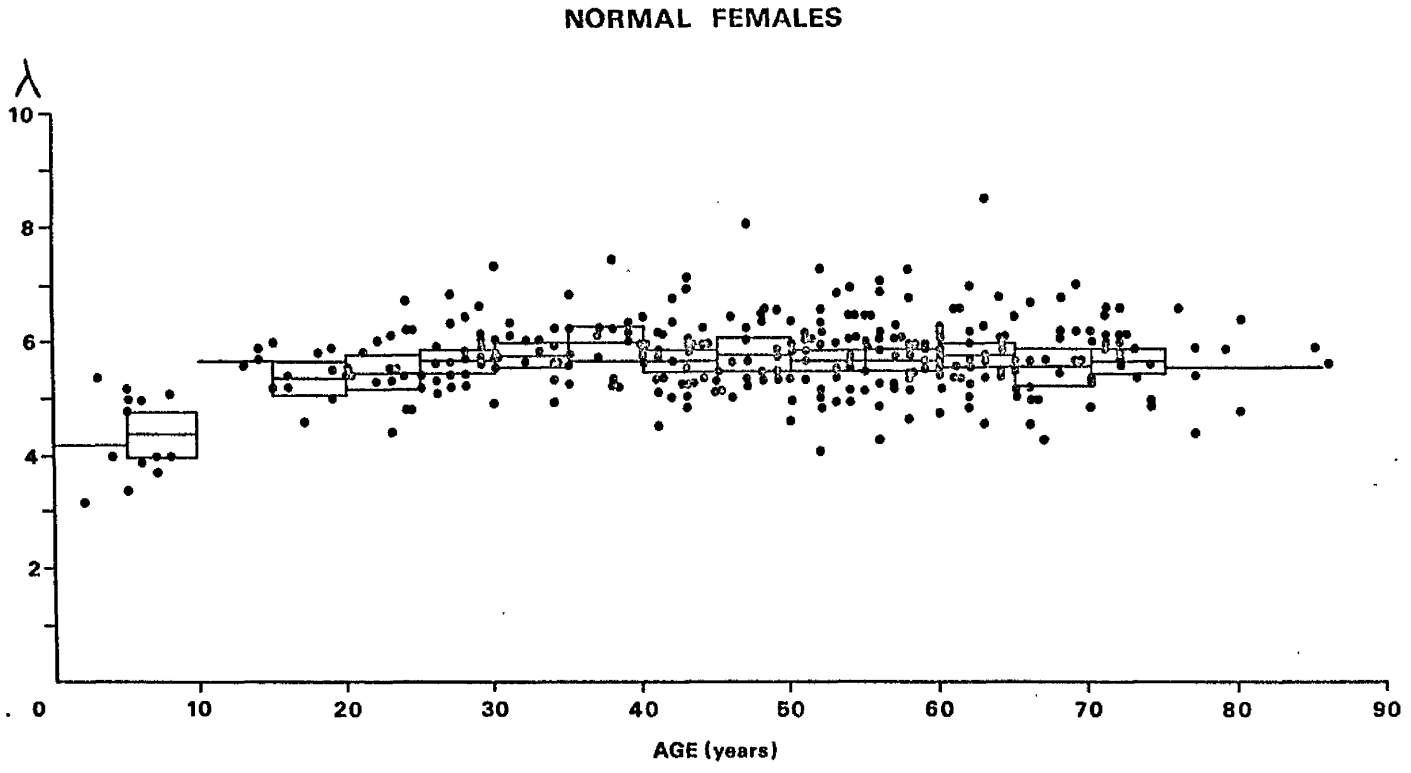


Fig 13 The relation between cortical density (λ) and age in 317 normal female volunteers (Group III). The cortical density for each individual and the mean and 2 S.E. range for each 5 year age group is indicated.

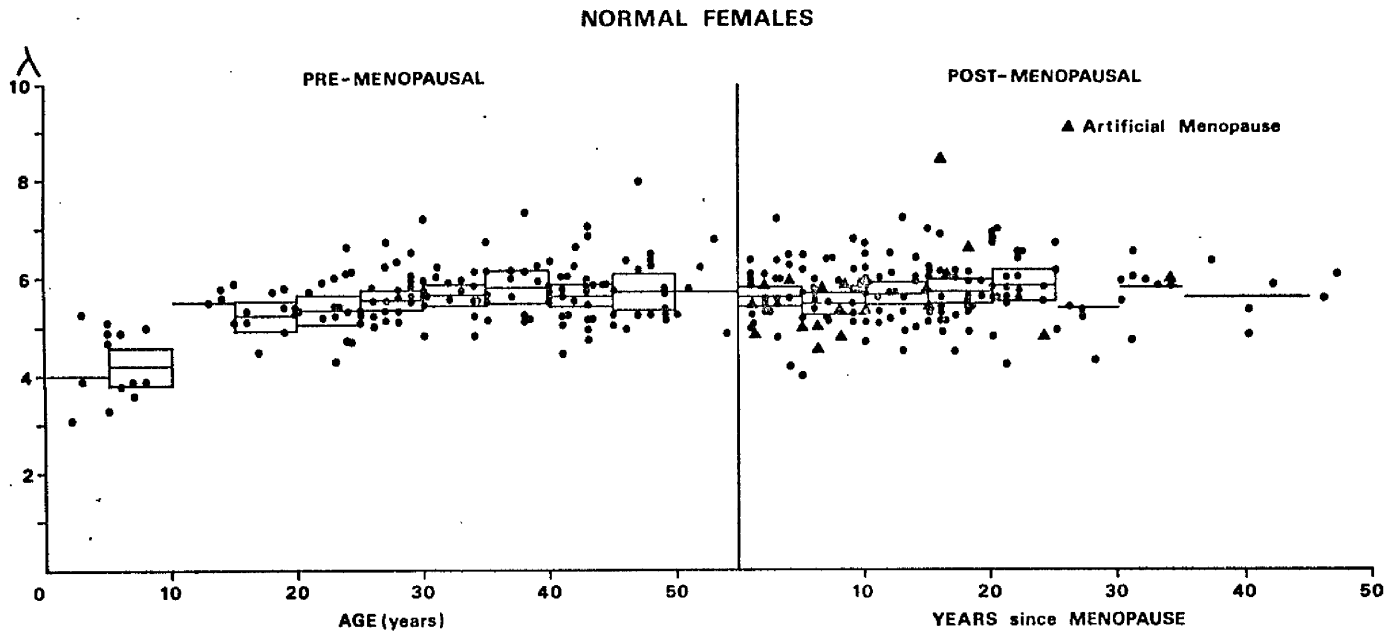


Fig 14 The cortical density (λ) of each female subject before the onset of the menopause, and the time since the onset of the menopause in the post-menopausal subjects is shown. (317 normal female subjects - Group III). No significant effect of the menopause on λ is detectable.

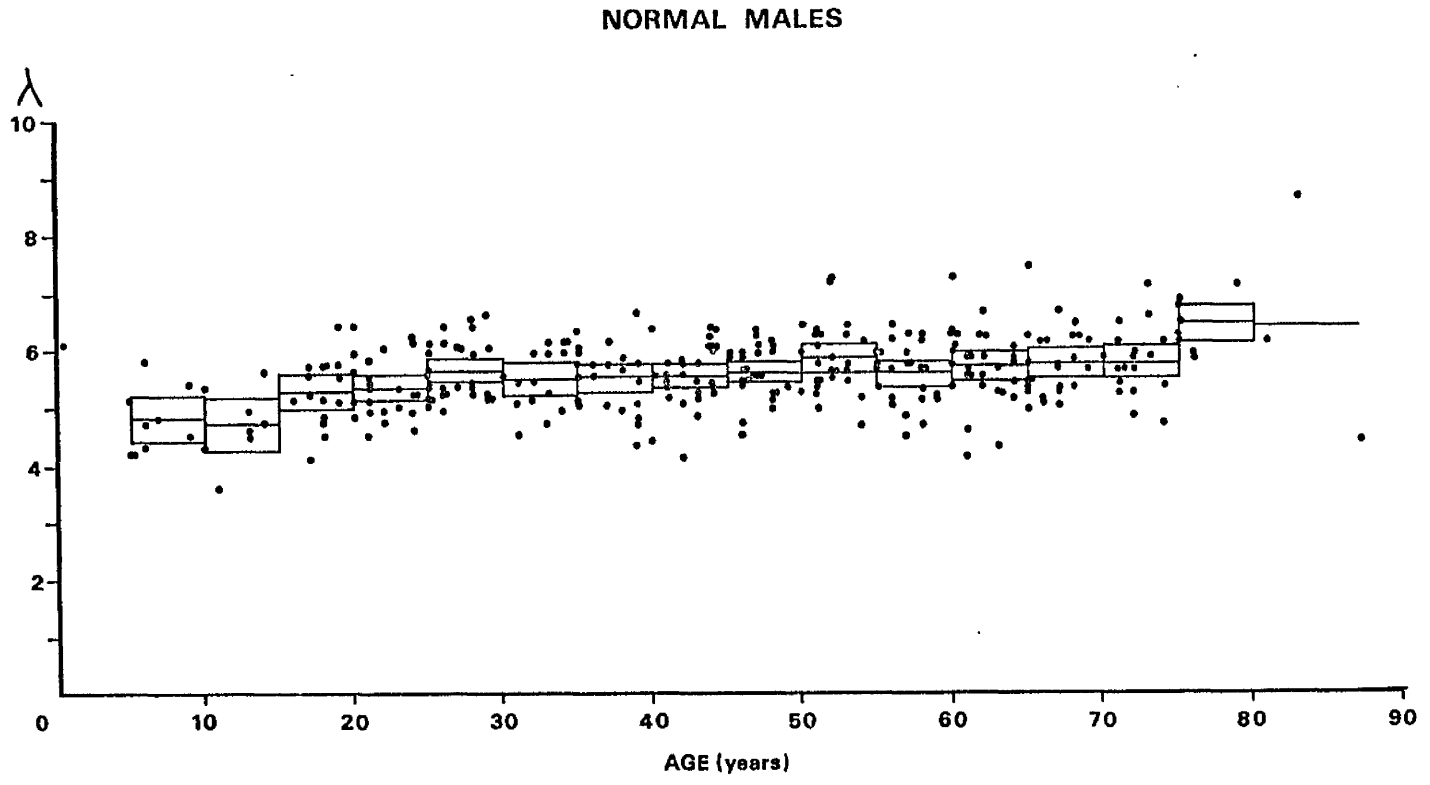


Fig 15 The change in cortical density (λ) of the 3rd metacarpal with age in 312 normal males (volunteers). The mean and 2 S.E. range for each 5 year age group is shown.

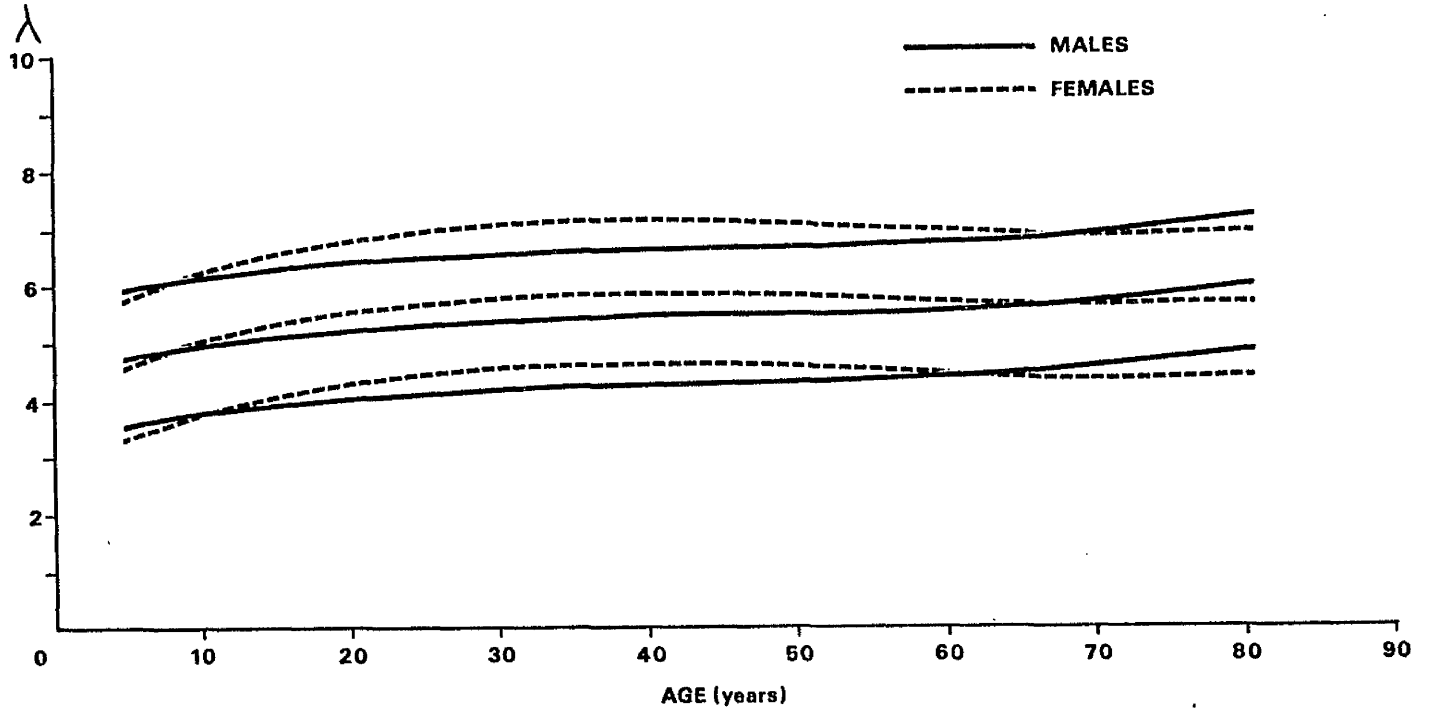


Fig 16 The change in cortical density (λ) with age comparing 312 normal male and 317 normal female subjects (Groups III and IV). The data is analysed in terms of a third order polynomial. The mean and the 5 and 95 percent limits are shown.

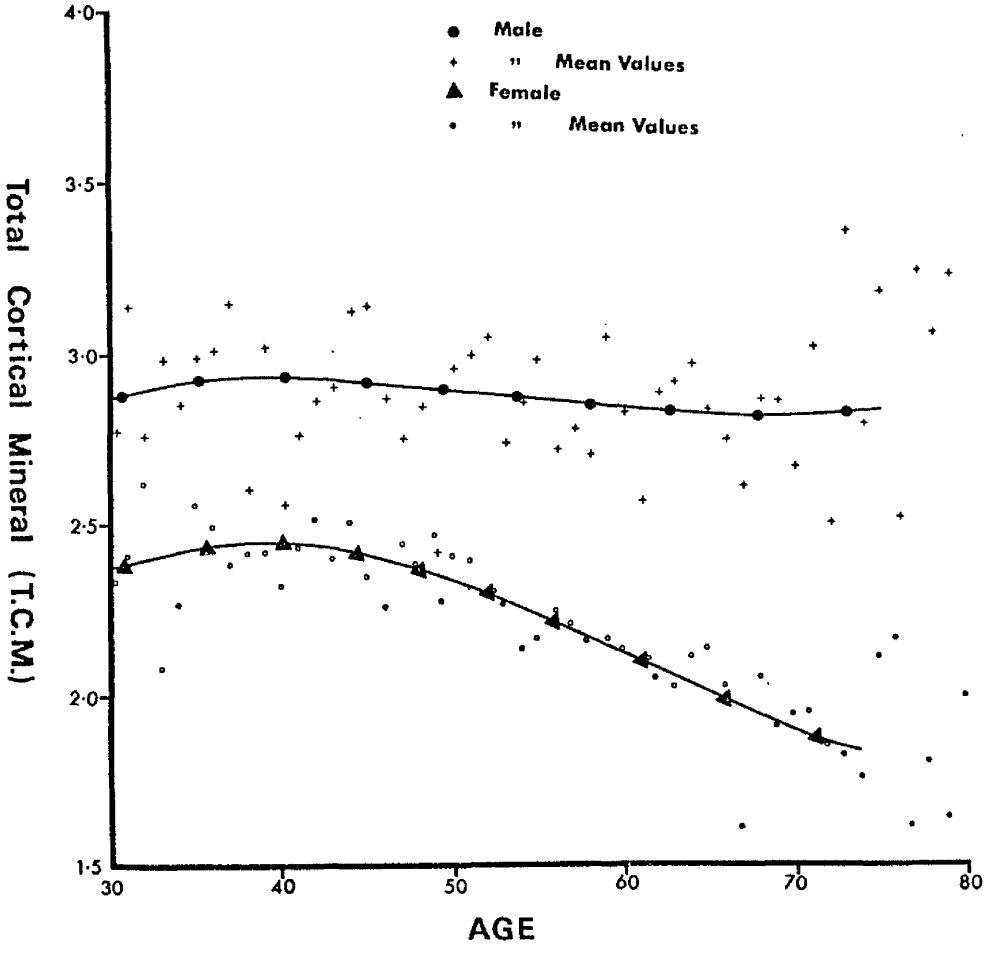


Fig 17 The change with age in the total cortical mineral of the 3rd metacarpal with age in normal male and female subjects (Groups III and IV). The mean for each year between 30 and 80 years is shown. The third order polynomial analysis is illustrated.

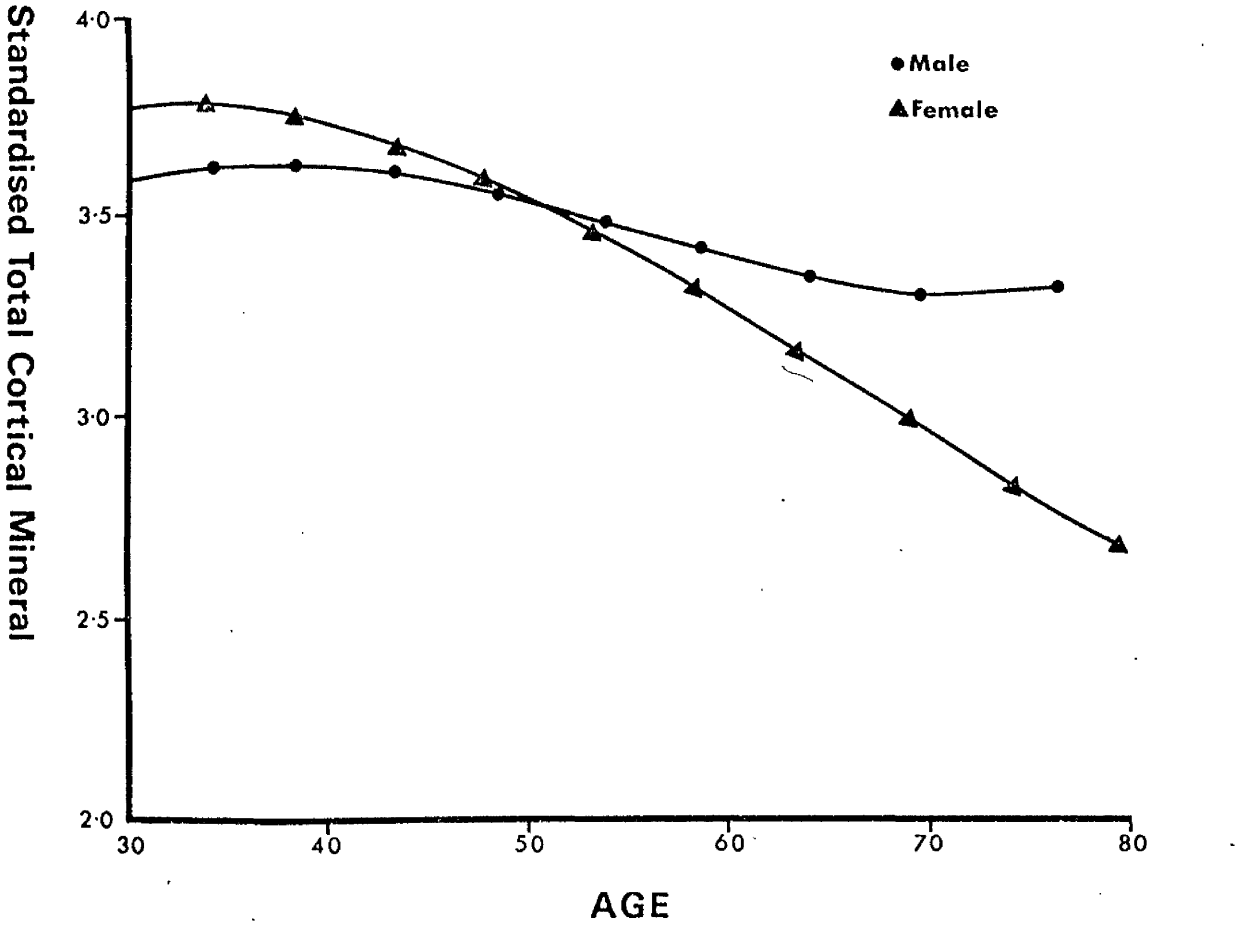


Fig 18 The change in the S.T.C.M. of the 3rd metacarpal with age in normal male and female subjects (Groups III and IV). The third order polynomial analysis is illustrated.

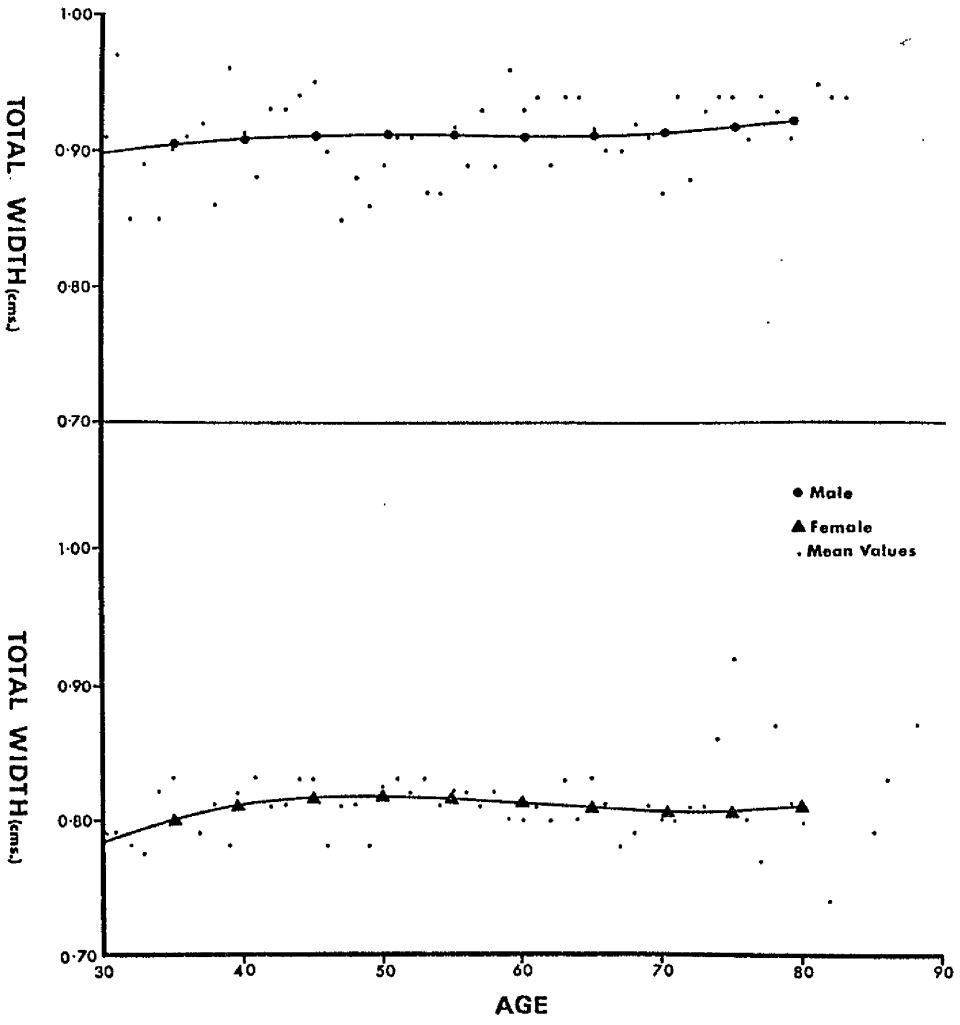


Fig 19 Changes in the total width of the 3rd metacarpal of normal male and female subjects with age (Groups III and IV). The third order polynomial analysis is shown.

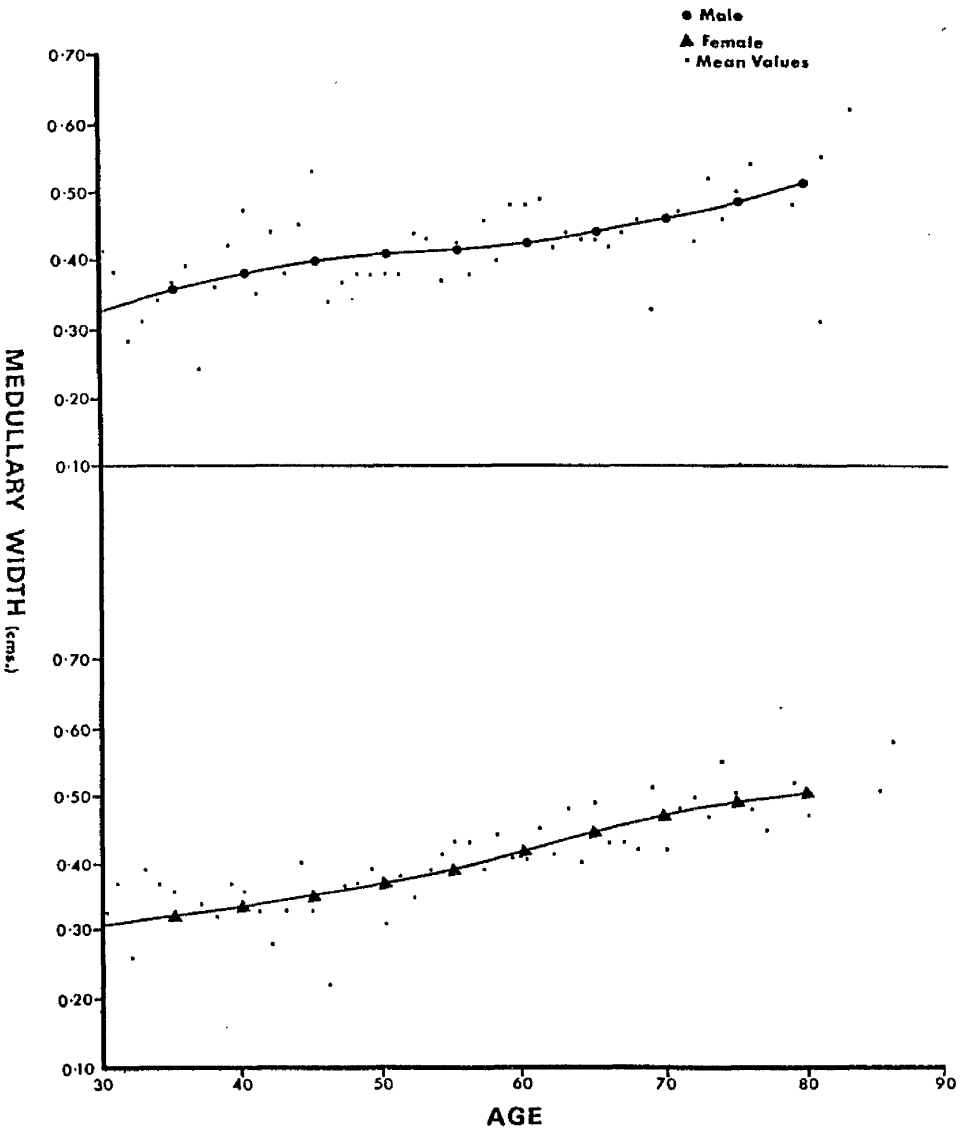


Fig 20 Changes in the medullary width of the 3rd metacarpal of normal male and female subjects with age (Groups III and IV). The third order polynomial analysis is shown.

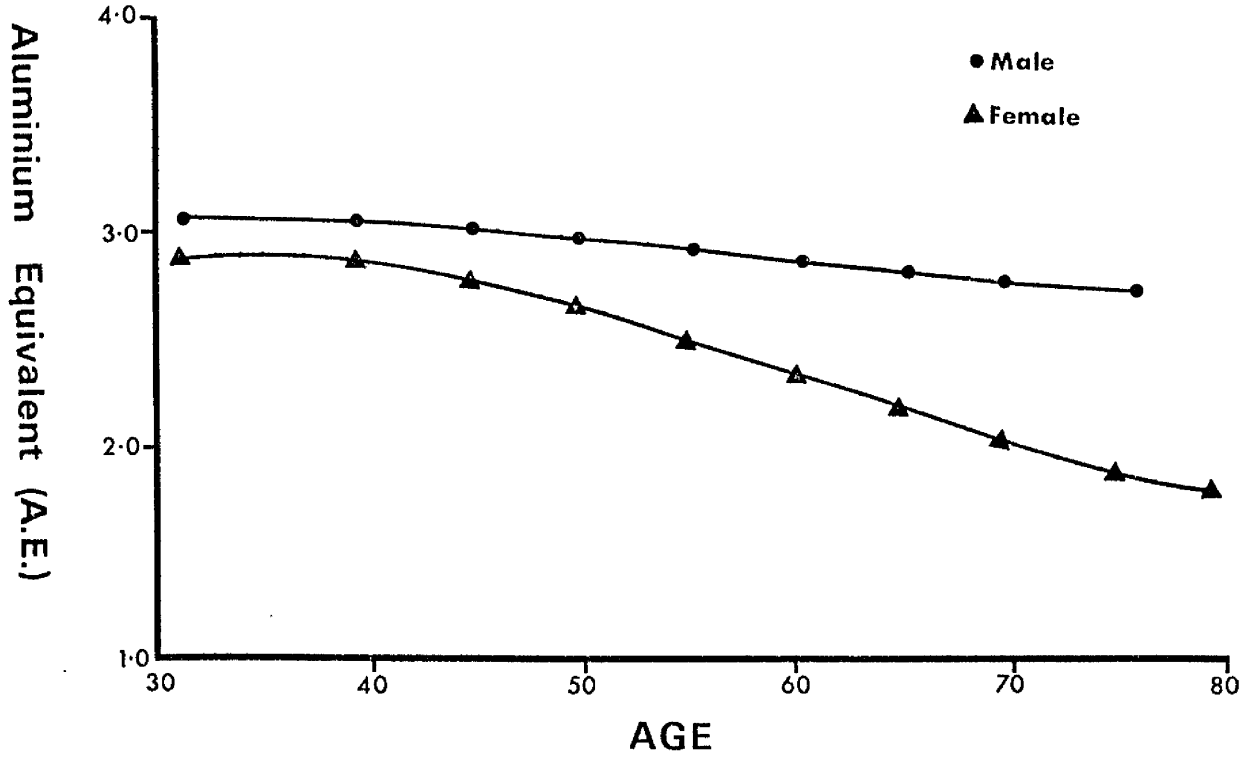


Fig 21 Changes in the aluminium equivalent of the 3rd metacarpal in normal male and female subjects with age. The data is analysed in terms of a third order polynomial.

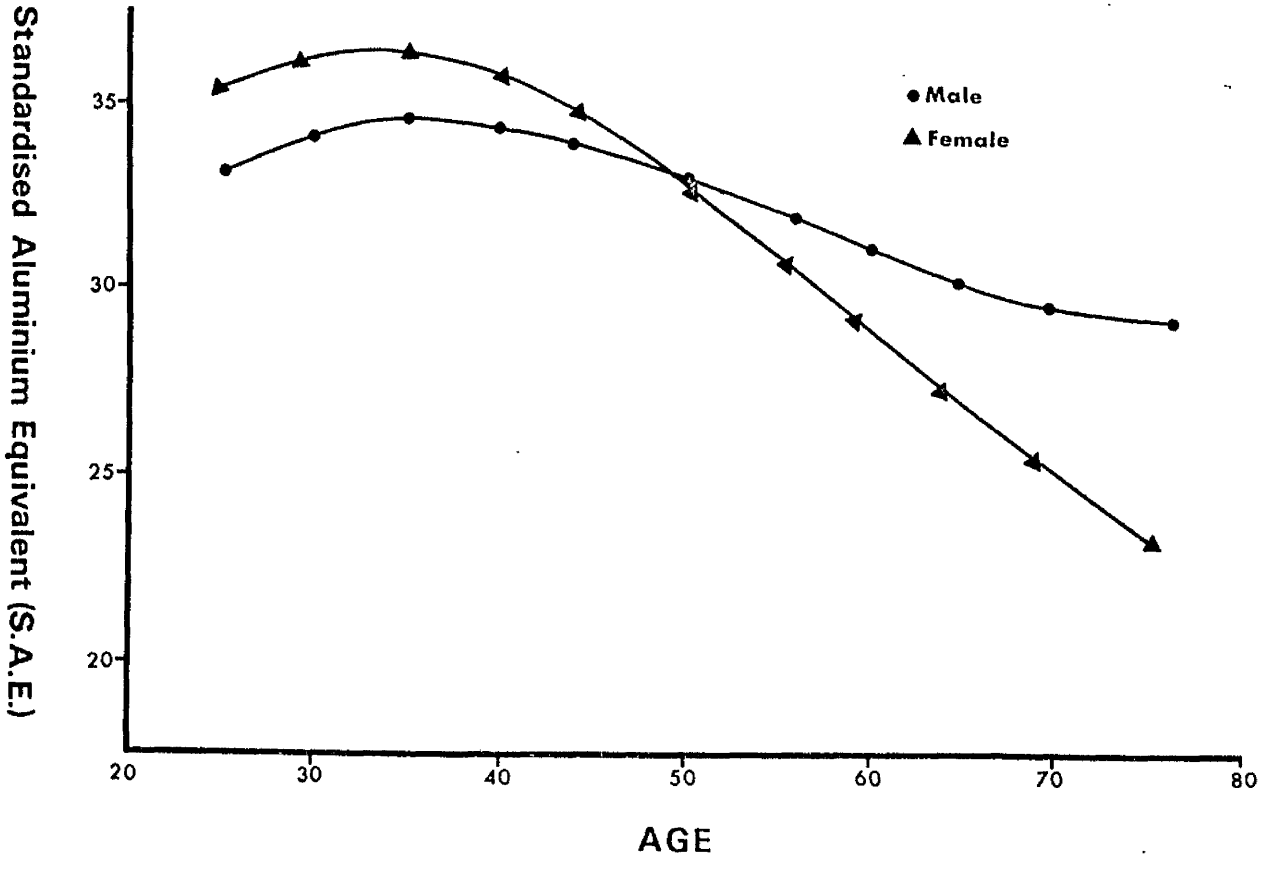


Fig 22 Changes in the S.A.E. with age in male and female subjects between 25 and 75 years shown as the mean values obtained by analysis in terms of a third order polynomial.

CHAPTER II

S.A.E. FEMALE SUBJECTS

Group	No. of subjects	< 5%	< 10%	< 20%	< 30%	< 40%	< 50%	> 50%
"Osteoporosis"	169	9.5	16.6	28.5	37.3	45.5	58.0	42.0
Fractured Femur	69	17.4	23.2	33.4	39.0	50.5	56.5	43.5
Steatorrhoea	18	27.7	39.0	55.5	74	78	83.5	16.5
Osteomalacia	8	12.5	37.5	50.0		62.5		37.5
Hyperparathyroidism	6	33.3			50	66.7		33.3
Hypoparathyroidism	6		33.3	50			66.7	33.3
Gastrectomy	3	66.5		100				

TABLE VI

The percentage of female patients in different diagnostic categories between the indicated percentile values derived by analysis of the S.A.E. in relation to age in the normal population (Group III).

S.A.E. MALE SUBJECTS

Group	No. of subjects	< 5%	< 10%	< 20%	< 30%	< 40%	< 50%	> 50%
"Osteoporosis"	26	18.6	22.3	33.5	55.5	78.0	83	17
Fractured Femur	25	19.2	34.5	50	57.5	61.5	65.5	34.5
Gastric surgery	86	11.4	16.1	35.6	51.5	64	74	26
Steatorrhoea	11	18.2	27.3	36.5	45.3		73	27
Osteomalacia	3	33.3	66.7	100				
Hyperparathyroidism	3		33.3	66.7				33.3

TABLE VII

The percentage of male subjects in different diagnostic categories between the indicated percentile values derived by analysis of the S.A.E. in relation to age in the normal population (Group IV).

		λ FEMALE SUBJECTS						
Group	No. of subjects	< 5%	< 10%	< 20%	< 30%	< 40%	< 50%	> 50%
"Osteoporosis"	169	1.0	14.8	26.0	34.2	40.1	50.7	49.3
Fractured Femur	69	10.0	17.2	27.1	35.7	47.1	50.5	49.5
Steatorrhoea	18	16.7	22.3	33.4	66.8	72.0	83.2	16.8
Osteomalacia	8	50.0		75.0		87.6		12.4
Hyperparathyroidism	6	33.3	50.0		66.6			33.4
Hypoparathyroidism	6						50.0	50.0
Gastrectomy	3			66.7				33.3

TABLE VIII

The percentage of female subjects in different diagnostic categories between the indicated percentile values derived by analysis of the cortical density (λ) in relation to age in the normal population (Group III).

Group	No. of subjects	λ MALE SUBJECTS < 5%	< 10%	< 20%	< 30%	< 40%	< 50%	> 50%
"Osteoporosis"	26	7.7	15.4	23.1	34.6	38.5	50.0	50.0
Fractured Femur	25	16.0	28.0	40.0		44.0	56.0	44.0
Gastric Surgery	86	7.0	12.8	18.6	28.0	38.5	54.4	45.6
Steatorrhoea	11	9.2		18.2		36.5		63.5
Osteomalacia	3	33.3			66.6		100	
Hyperparathyroid	3		33.3		66.6			33.4

TABLE IX

The percentage of male subjects in different diagnostic categories between the indicated percentile values derived by analysis of the cortical density (λ) in relation to age in the normal population (Group IV).

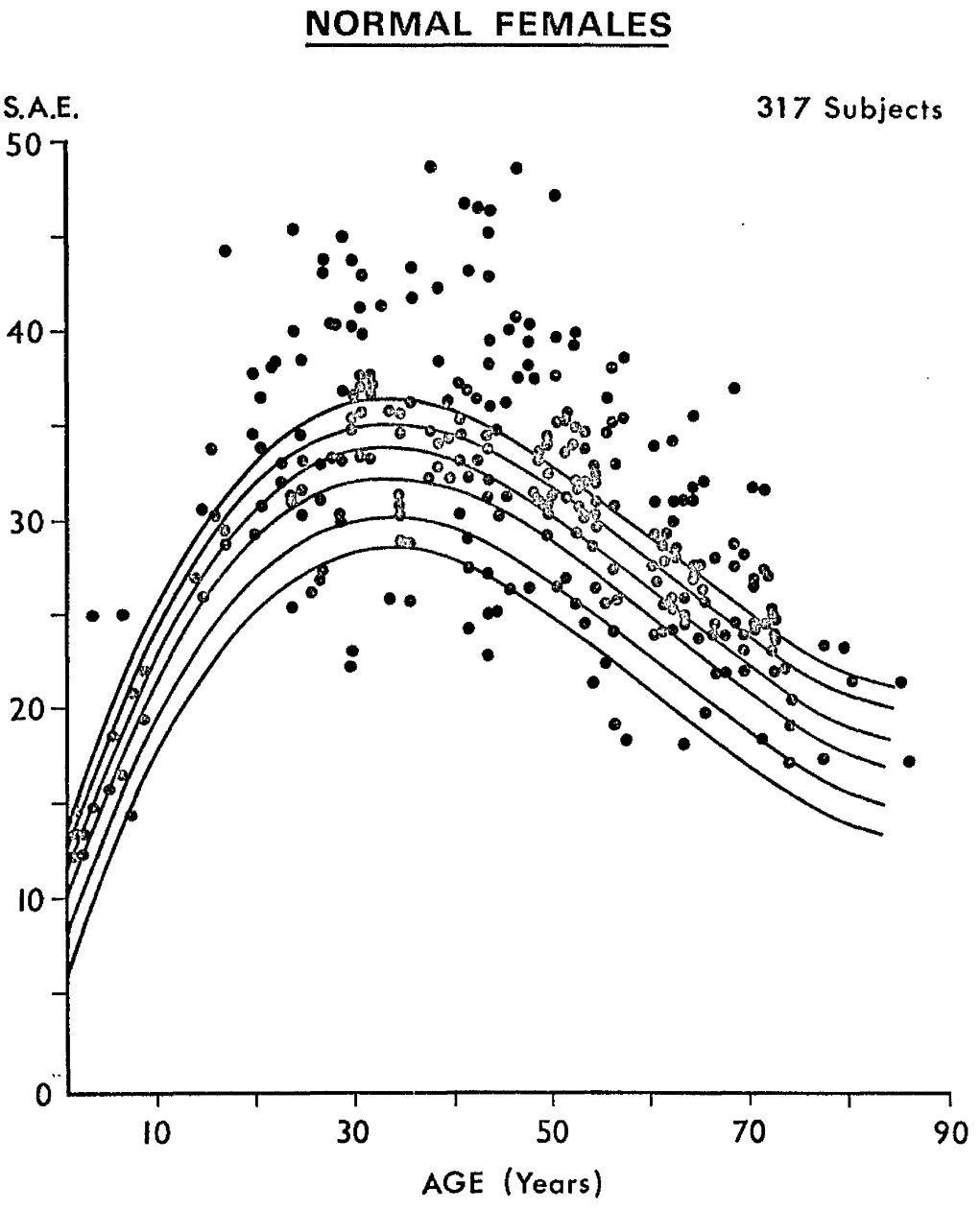


Fig 23 The 5, 10, 20, 30, 40 and 50 percentile values for the S.A.E. plotted against age derived from the 317 normal female subjects of Group III.

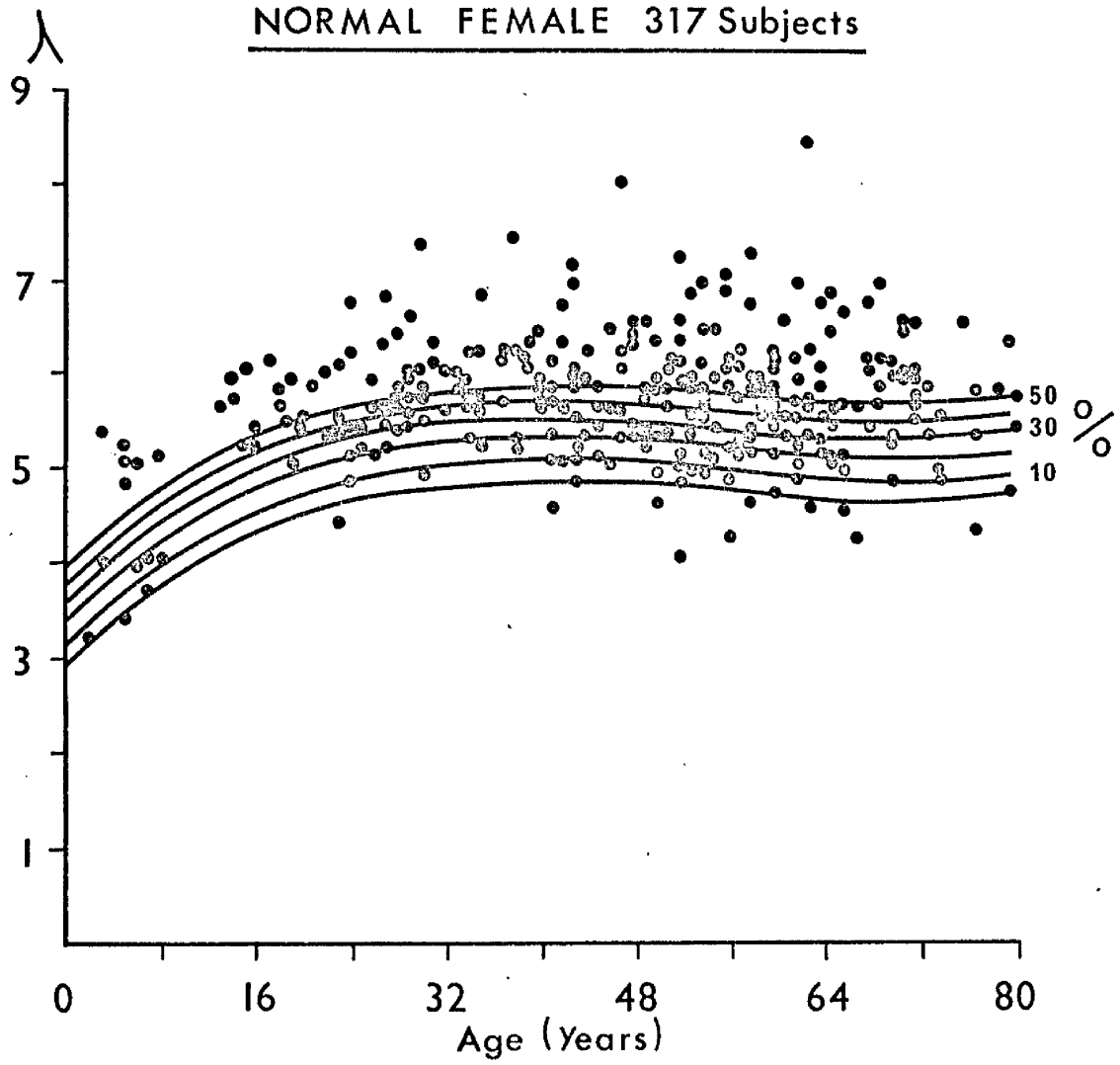


Fig 24 The percentile values from 5 to 50 between 5 and 80 years derived from the cortical density (λ) measured in 317 normal female subjects (Group III).

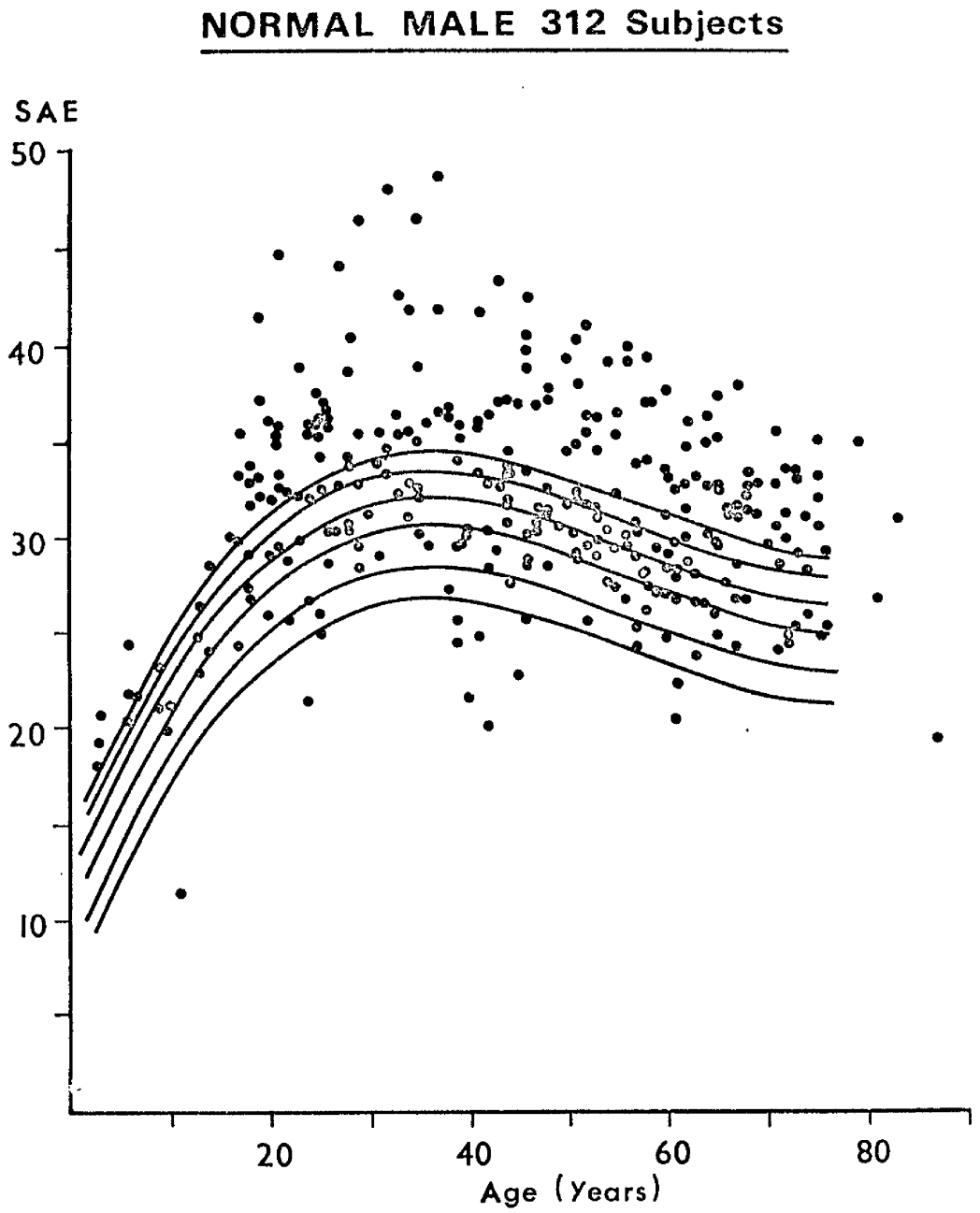


Fig 25 The percentile values between 5 and 50 years for the whole bone density (S.A.E.) in normal male subjects (Group IV - 312 subjects) against age.

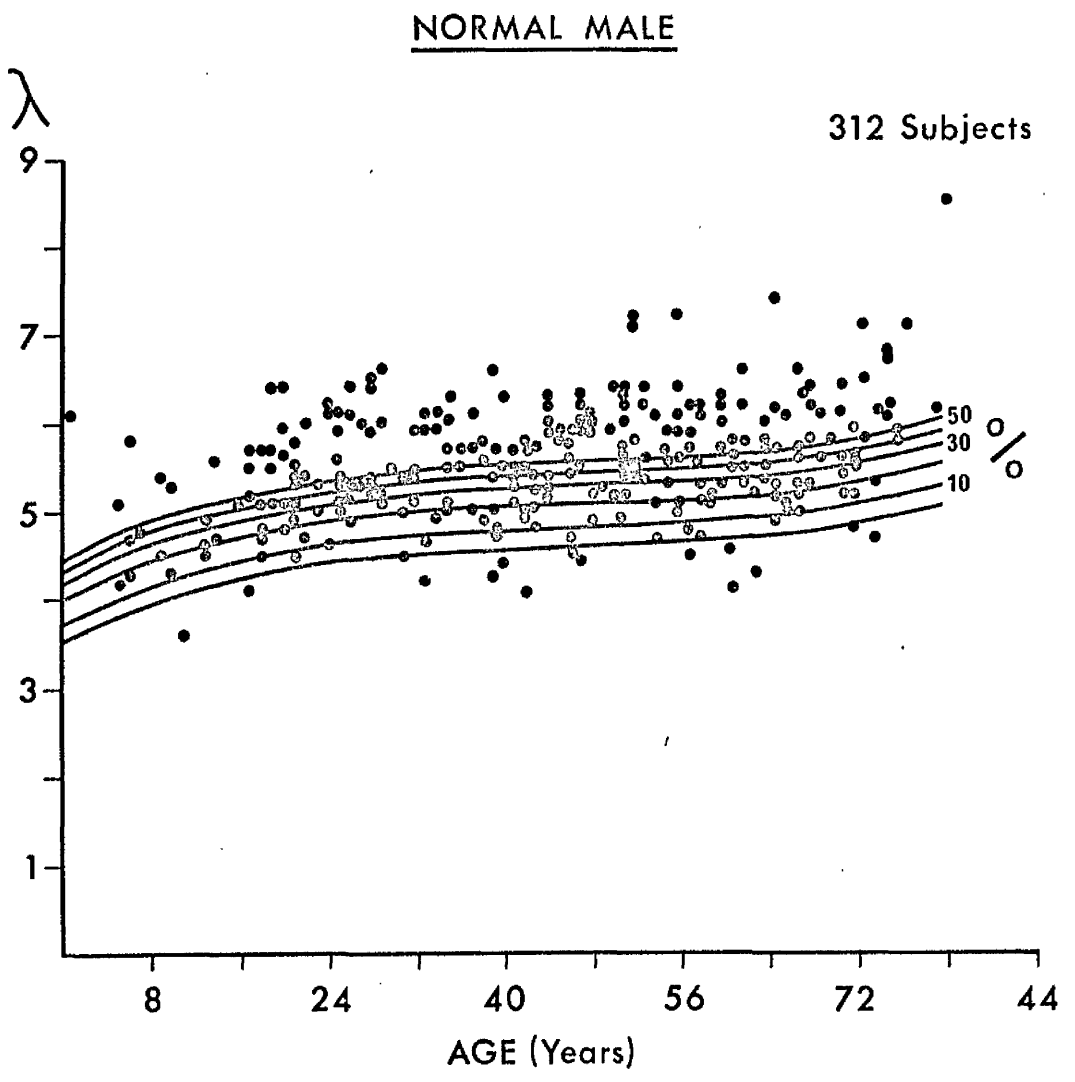


Fig 26 The percentile values for cortical density derived from 312 normal male subjects (Group IV) plotted against age.

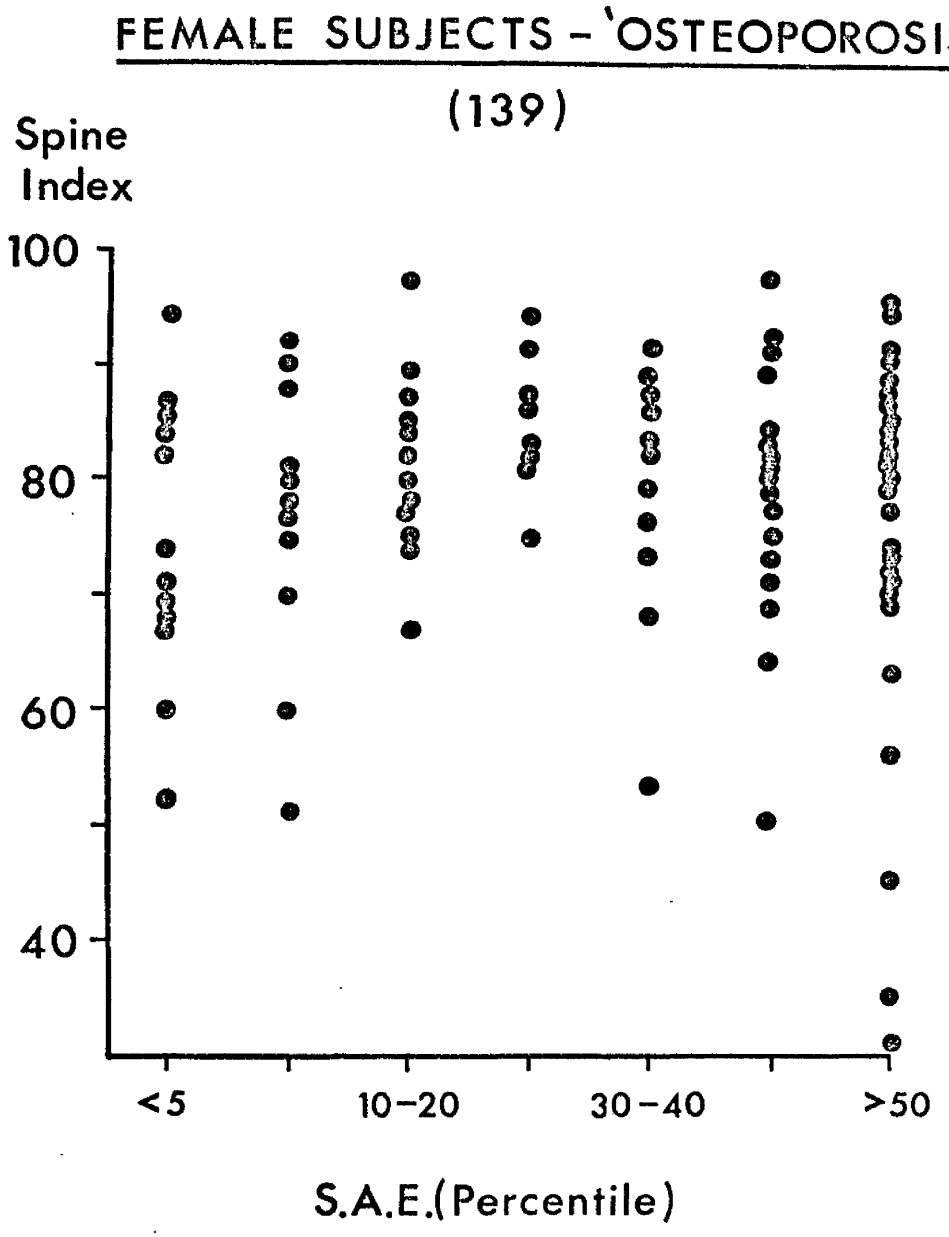


Fig 27 The Spinal Index plotted against the percentile value for S.A.E. (of the 3rd metacarpal) in female 'osteoporotic' patients. A Spinal Index below 80 indicates significant biconcavity. It is evident that spinal biconcavity may be severe even when the patient is above the mean value for her age (i.e. above the 50 percentile).

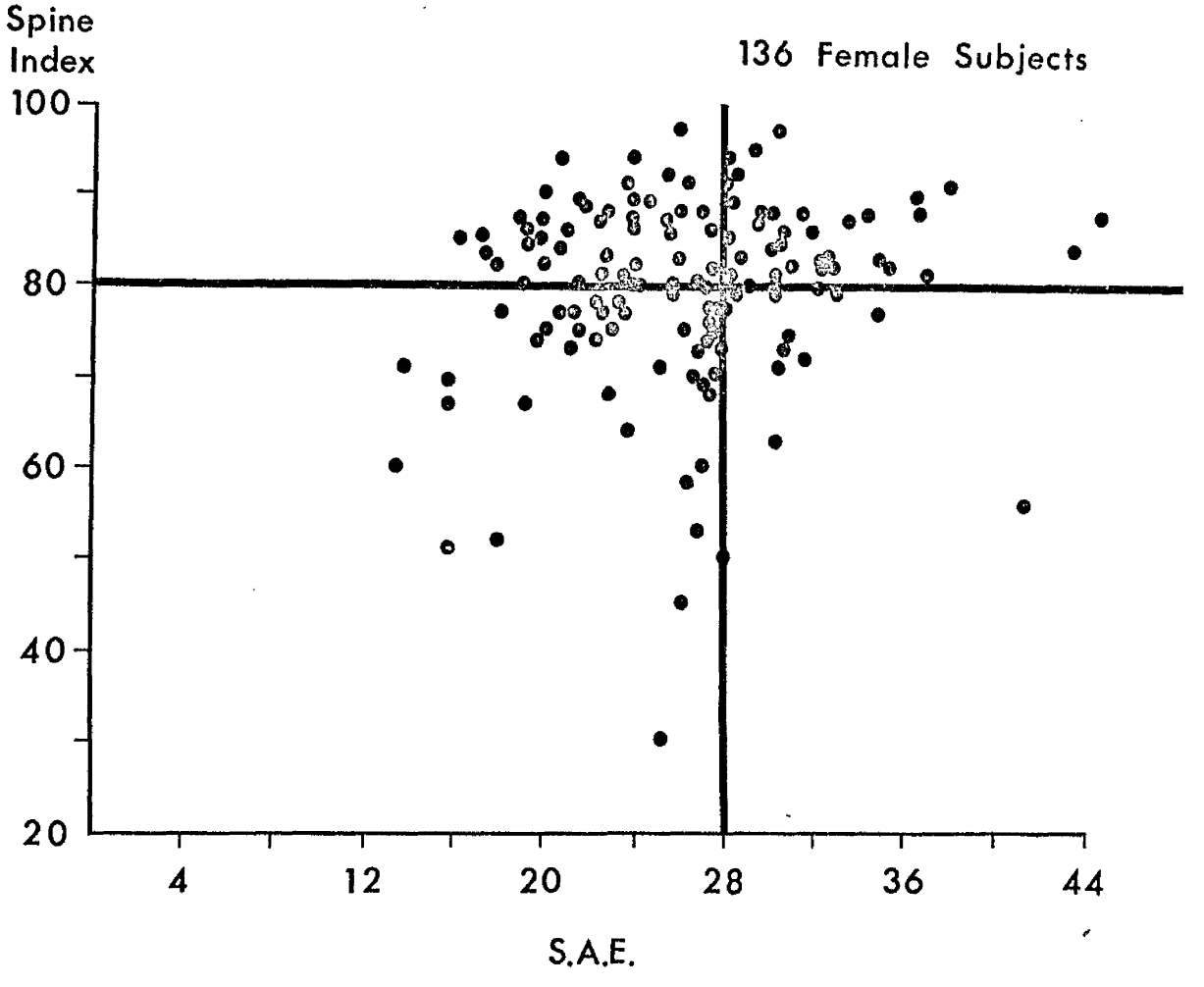


Fig 28 The Spinal Index plotted against the whole bone density (S.A.E.) of the same subjects illustrated in Fig 25. There is a much higher incidence of spinal biconcavity below an S.A.E. of 28 (the 5 percentile value at 35 years of age when the whole bone density is at its greatest in women).

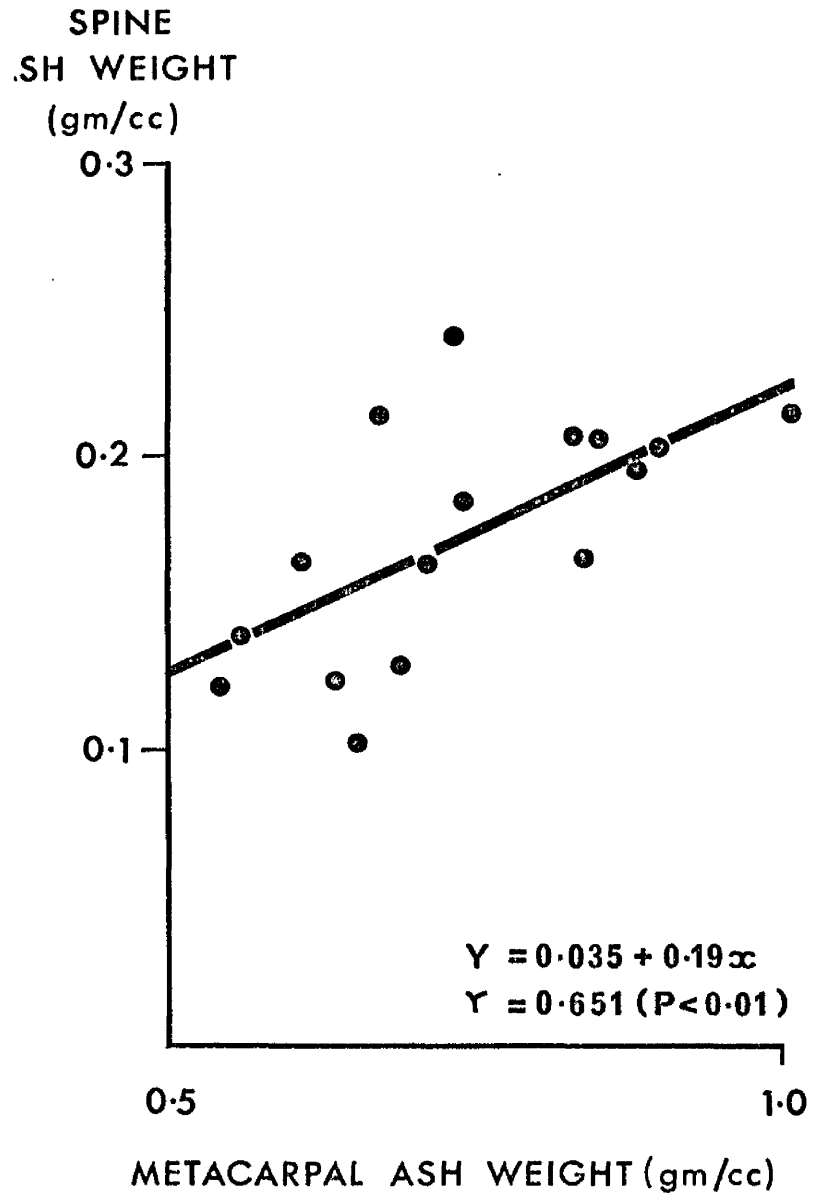


Fig 29 The spinal ash weight (gm per cc) plotted against metacarpal ash weight (gm per cc). The bone was derived from post-mortems on patients who died of conditions not associated with metabolic bone disease.

CHAPTER III

TABLE X

The ratios of the rates of mineral loss in male and female subjects at age 50 years.

		A.E.	S.A.E.	T.C.M.	S.T.C.M.
Ratio	$\frac{\text{Male}}{\text{Female}}$	0.43	0.48	0.12	0.33

TABLE XI

Increase in prevalence of bone mineral loss as measured by the various parameters in male and female subjects between 35 and 75 years.

		A.E.	S.A.E.	T.C.M.	S.T.C.M.
Percentage increase	Male	132	123	26	88
	Female	306	257	217	268

TABLE XII

Categorisation of Fracture Sites

GROUP A (Industrial)	GROUP B (Home)	GROUP C 'Osteoporotic'	Uncategorised
Phalanges (hands and feet)	Spine	Clavicle	Ribs
Tarsals and metatarsals	Pelvis	Fibula (malleolus)	
Carpals and metacarpals		Fibula (head and shaft)	
Radius - upper end		Tibia (malleolus)	
		Tibia (shaft)	
		Tibia (head)	
		Patella	
		Femur (shaft)	
		Femur (neck)	
		Femur (trochanter)	
		Radius (lower end)	
		Humerus (upper end)	
		Humerus (rest)	
		Ulna	

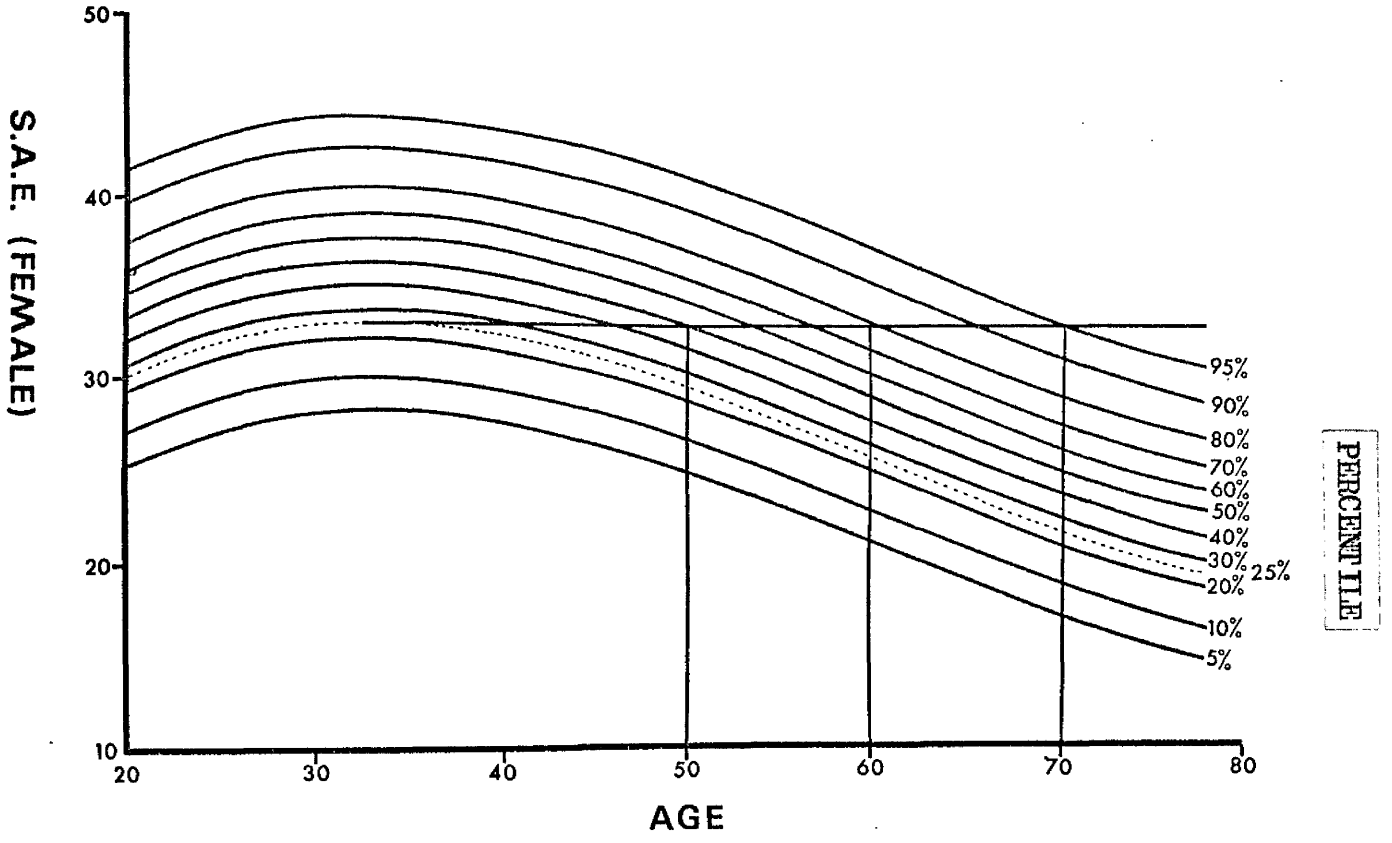


Fig 30 This figure illustrates the derivation of the increasing prevalence of whole bone density below the 25 percentile at 35 years. The percentile values between 5 and 95 in the female subjects are shown. A line parallel to the 'x' axis has been arbitrarily drawn from the 25 percentile value at the age of 35. The rate of change of whole bone density can be determined from the intercept of this line with the percentile values. The rates of change vary with age and are illustrated for each X-ray measurement in Fig 31.

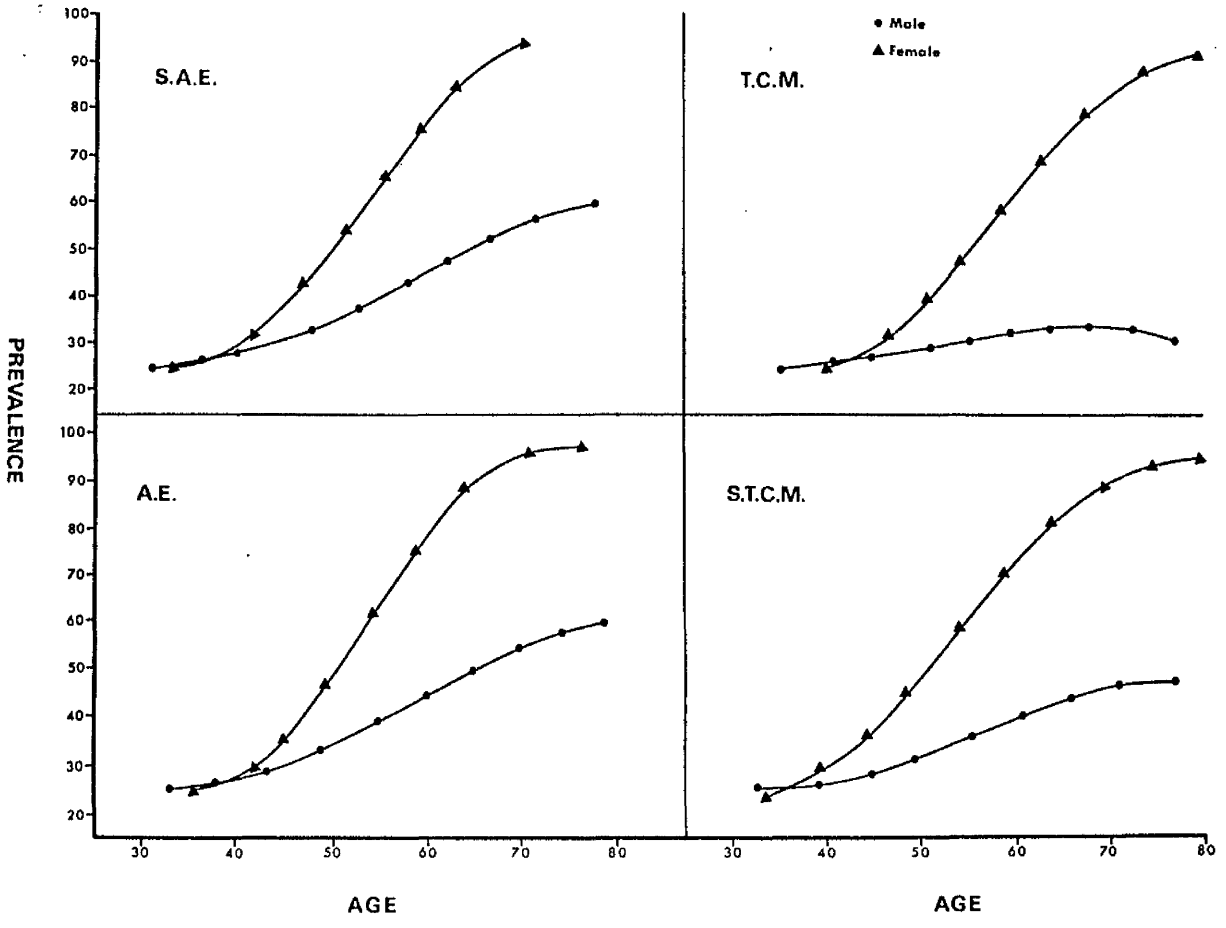


Fig 31 The changing prevalence of low bone mass with increasing age determined for the A.E., S.A.E., T.C.M. and S.T.C.M. (see Fig 30). This prevalence is a function of age, sex and the type of X-ray measurement used to determine the various parameters of bone mass.

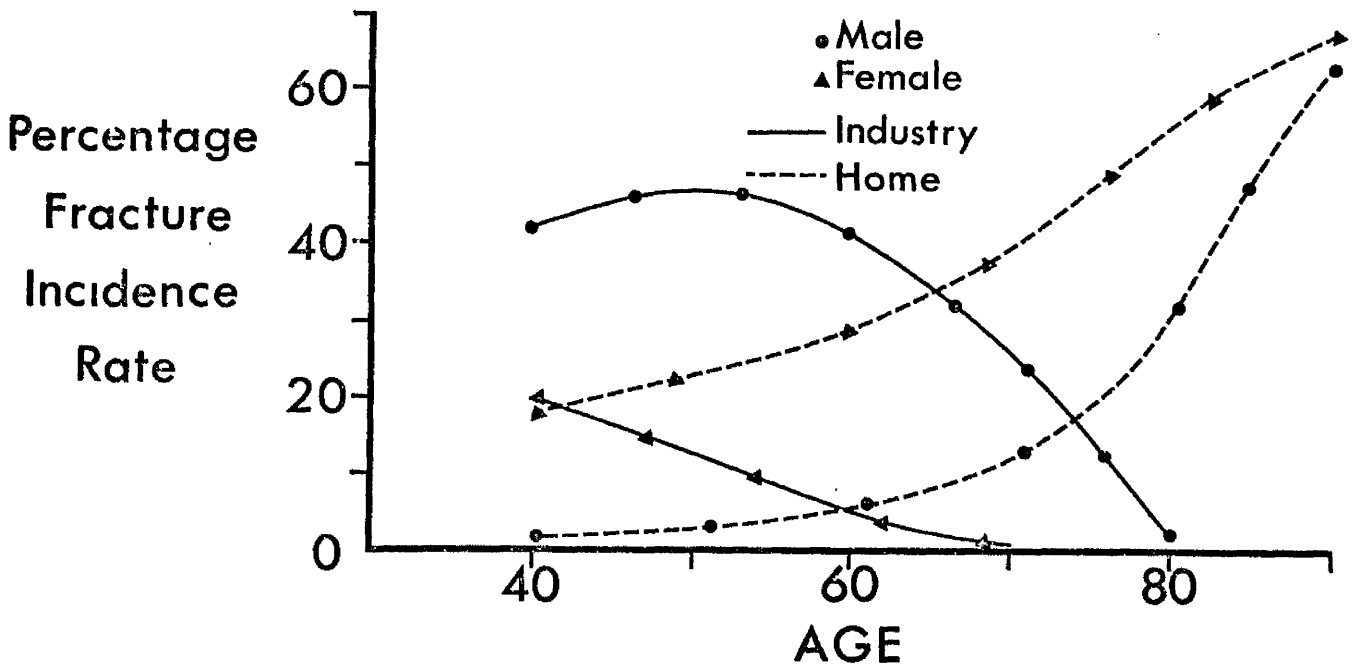


Fig 32 The changing percentage fracture incidence rate plotted against age for accidents in the home and at work for both men and women.

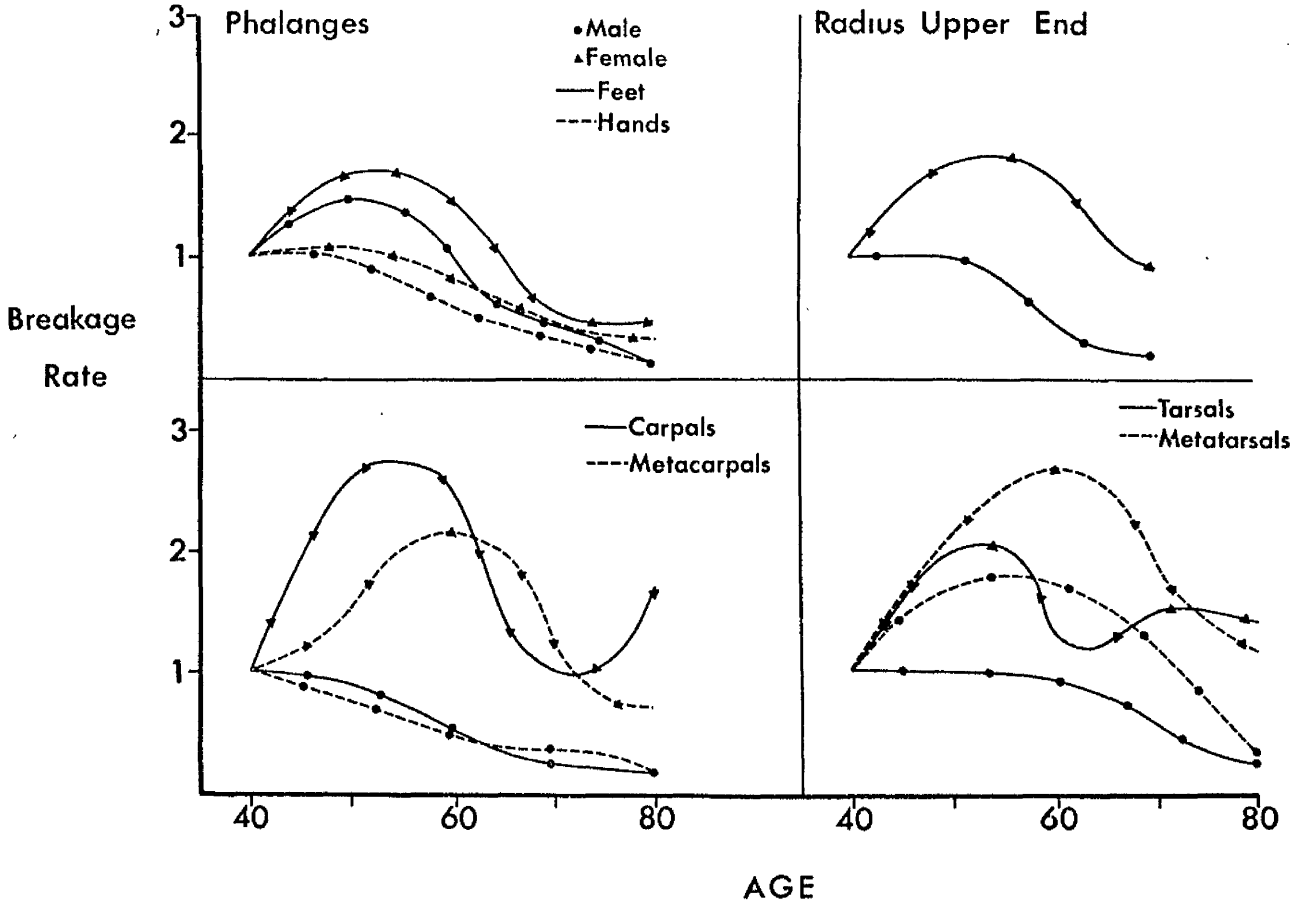


Fig 33 The changing fracture incidence with age in male and female subjects. The graphs do not show the absolute changes, but illustrate the change in incidence relative to the incidence at the age of 40 years. The resemblance to fractures sustained in industry are clear. The fracture sites are those designated as Group A (see Table XII and Fig 32).

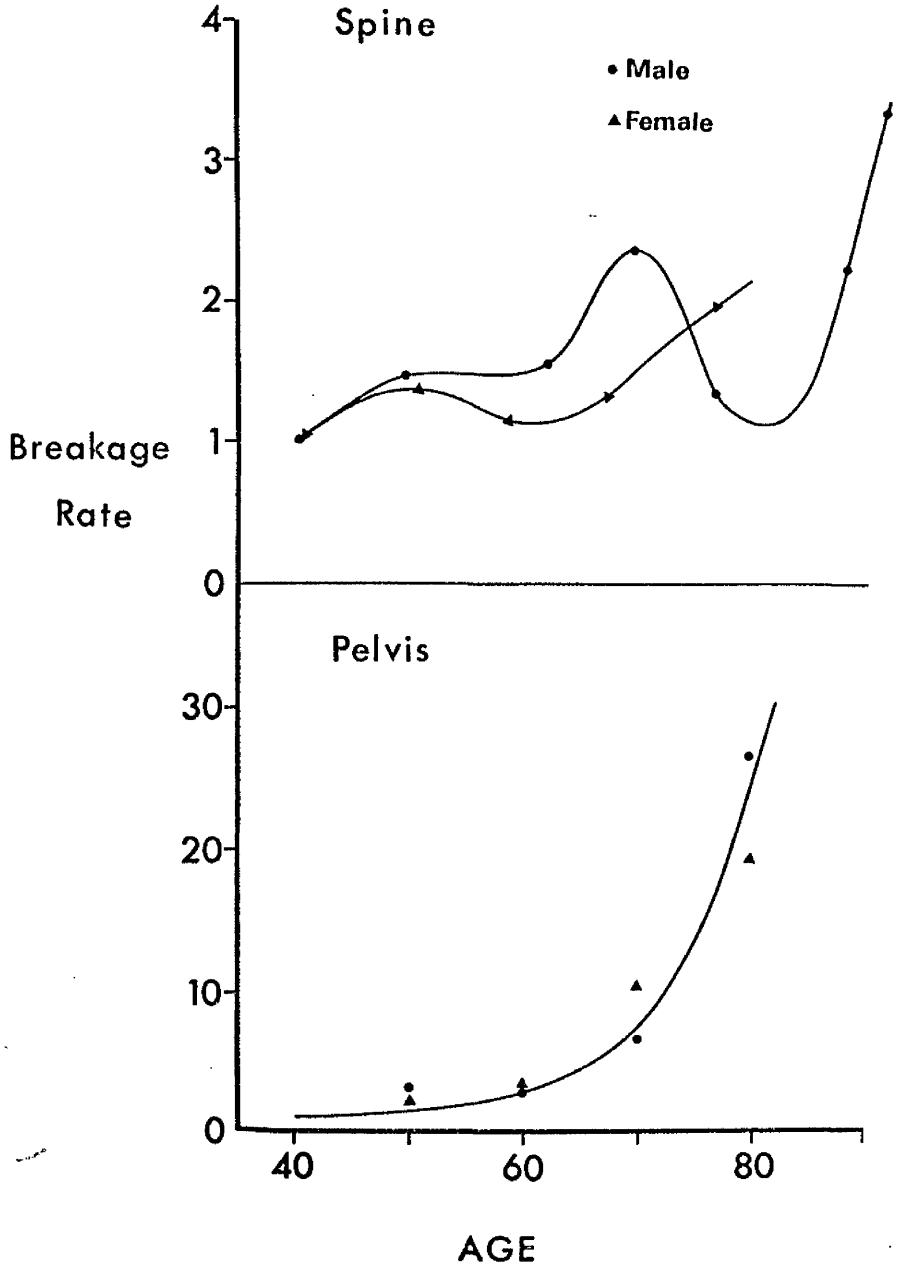


Fig 34 The changing fracture incidence of spine and pelvis relative to the incidence at the age of 40 years in both male and female subjects. These fractures belong to Group B (Table XII) and resemble the changing incidence of fractures with age sustained in the home (see Fig 32).

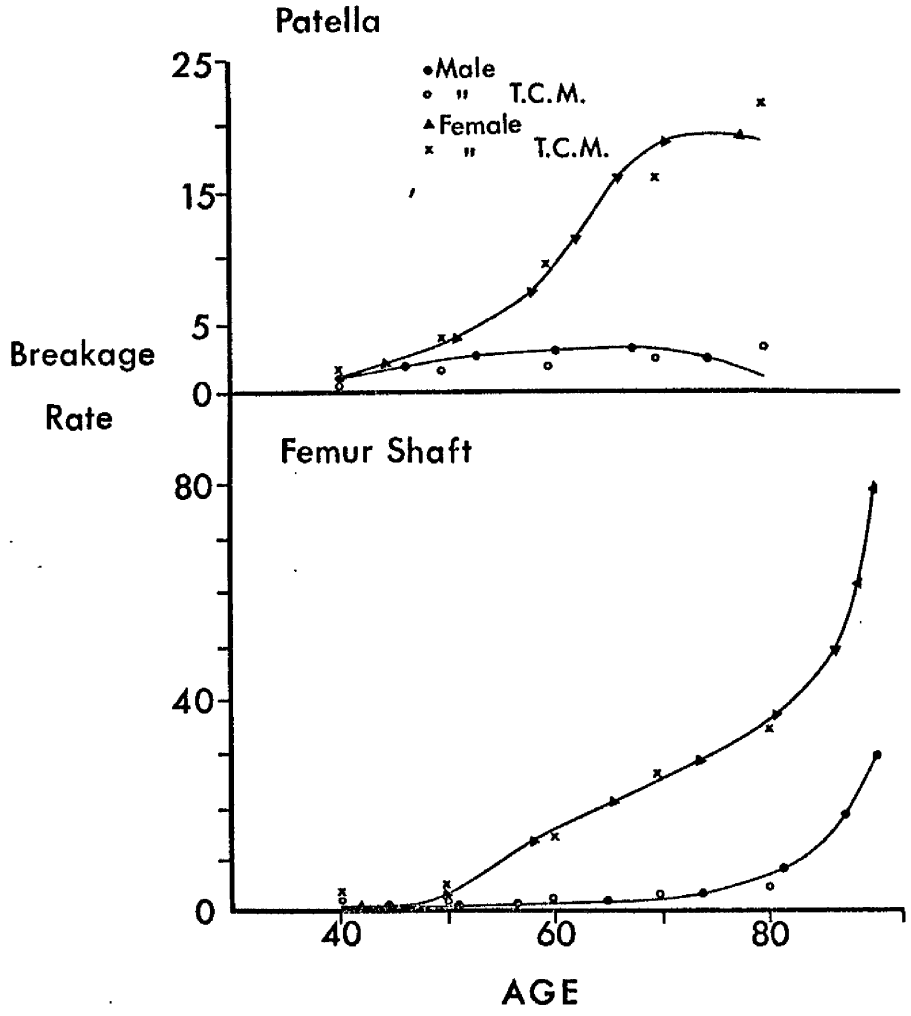


Fig 35 The changing fracture incidence in the patella and femoral shaft in male and female subjects with age. The changing incidence between 40 and 75 years very closely follows the rate of change in the incidence of total cortical mineral below the 25 percentile in the normal male (0) and female subjects (X). The fracture sites belong to Group C (Table XII). The fracture incidence rises markedly for the female subjects but shows little change with age in the male subjects. Above the age of 75 years the incidence of fracture in both the male and female subjects rises sharply and is no longer related to the rate of change of T.C.M. with age.

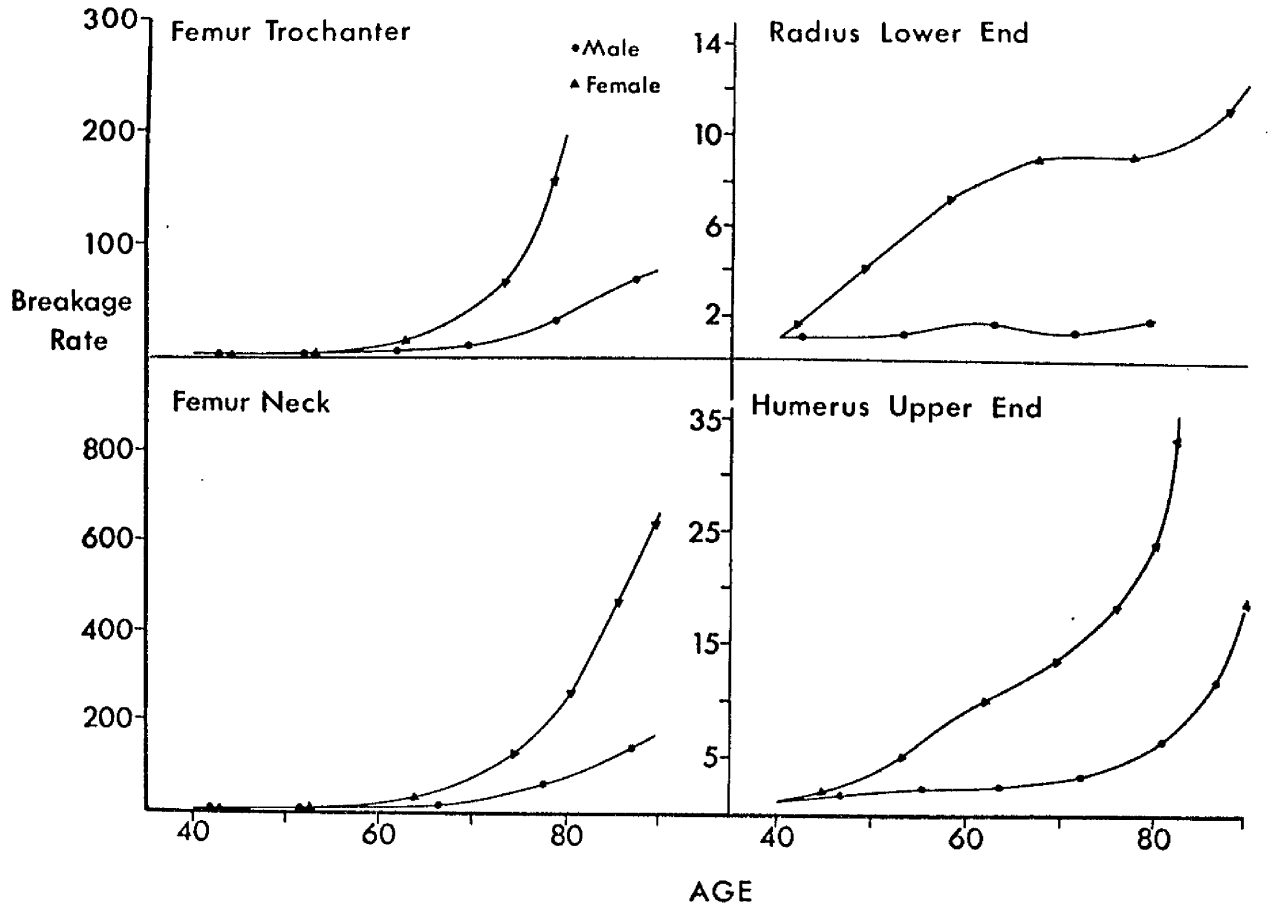


Fig 36 The pattern of change in fracture incidence of fracture in male and female subjects with age of the intertrochanteric fractures of femur, lower end of radius, femoral neck and upper end of humerus, is similar to those in Fig 35. These fractures again belong to Group C, and the relative rates of change up to 75 years of age are very similar to the changes in T.C.M. with age (see Table XII).

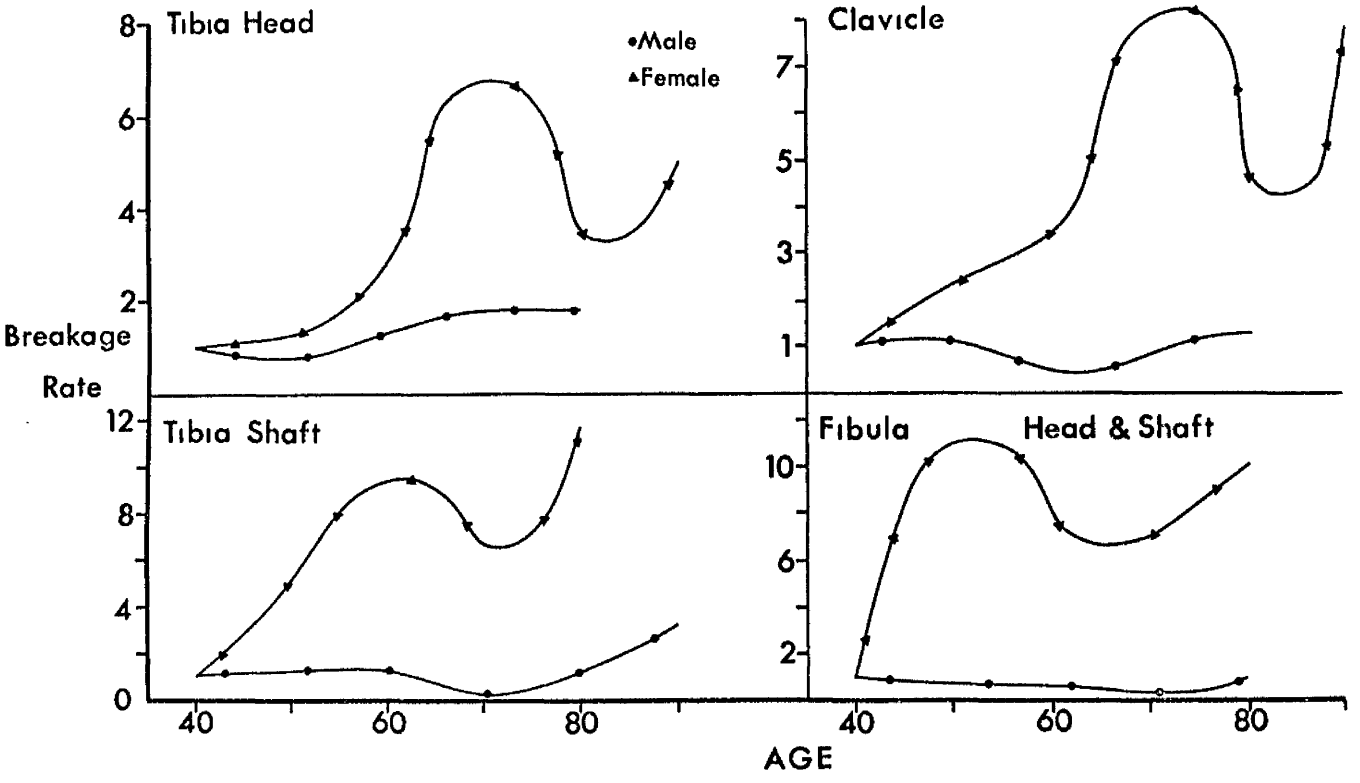


Fig 37 The changing fracture incidence with age relative to the incidence at the age of 40 years. Again the pattern of change is very similar to the T.C.M. as illustrated for the patella and femoral shaft in Fig 35. The incidence of fracture changes little for male subjects between 40 and 75 years. There is a marked rise in the fracture incidence in female subjects between 40 and 75 years. Both sexes again show the increase in incidence after the age of 75 to 80 years. These fracture sites are included in Group C (Table XII).

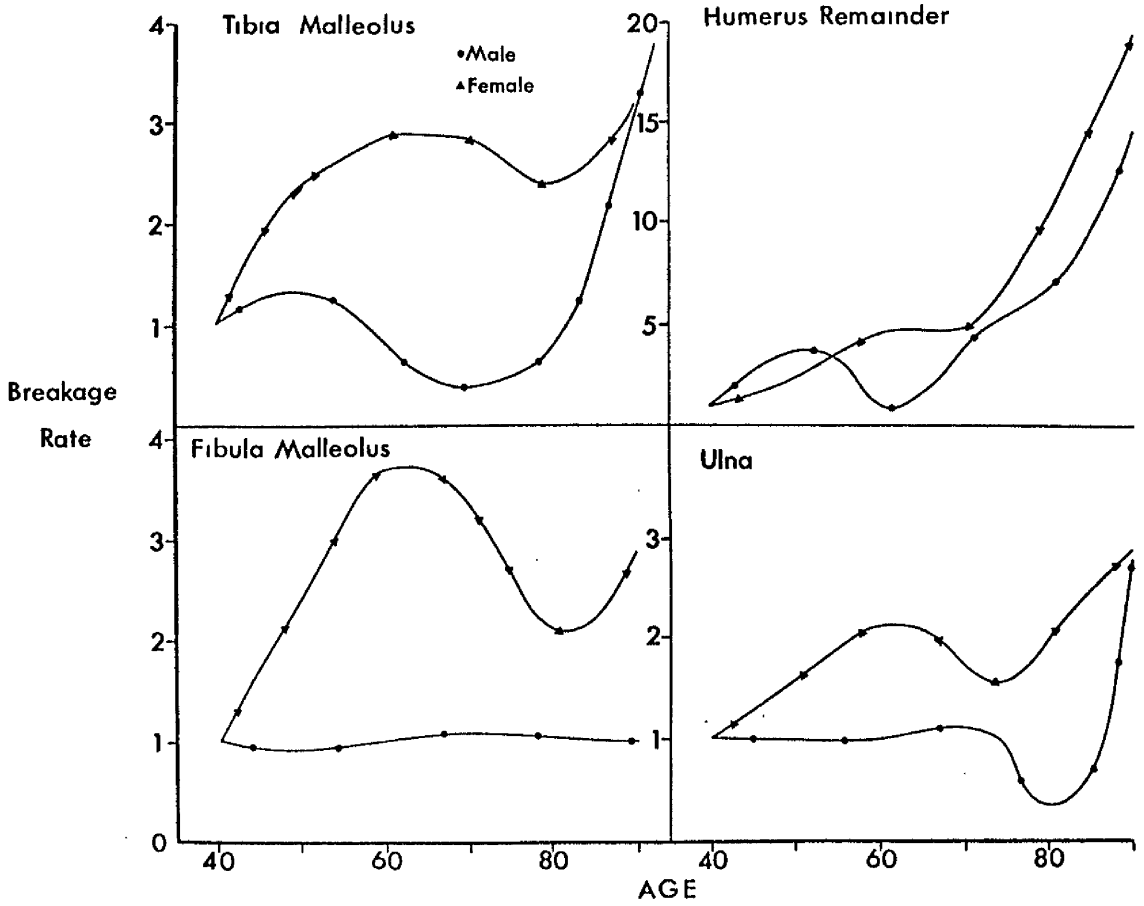


Fig 38 Fracture sites included in Group C (Table XII). Again the relatively small change in bone breakage rate in the male subjects below 75 years is in contrast to the change in female subjects, resembling the changes in T.C.M. with age (Figs 31 and 35).

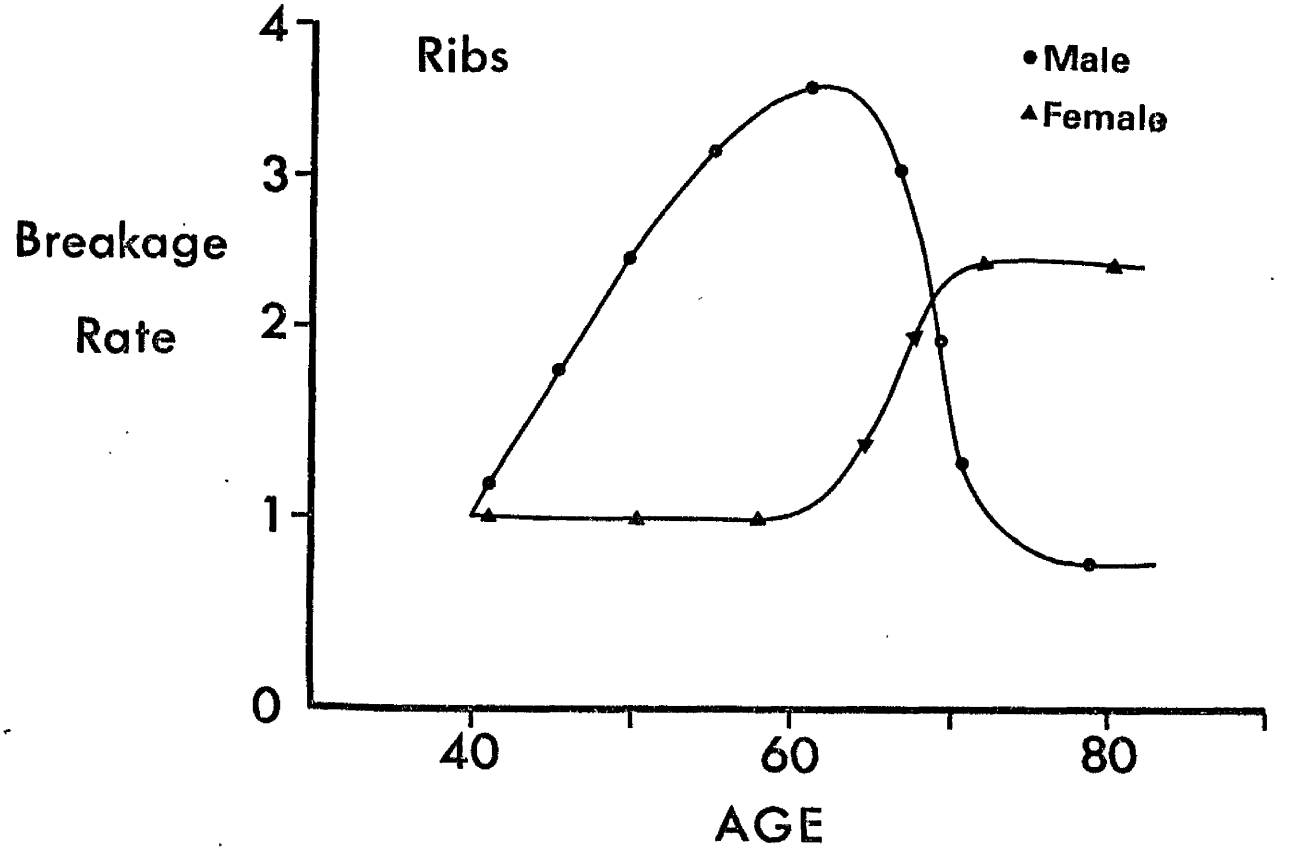


Fig 39 Fractures of the ribs show a change in incidence of fracture with age which does not fall into any of the patterns A, B or C and is entered separately under D (Table XII).

CHAPTER IV

CALCIUM ABSORPTION

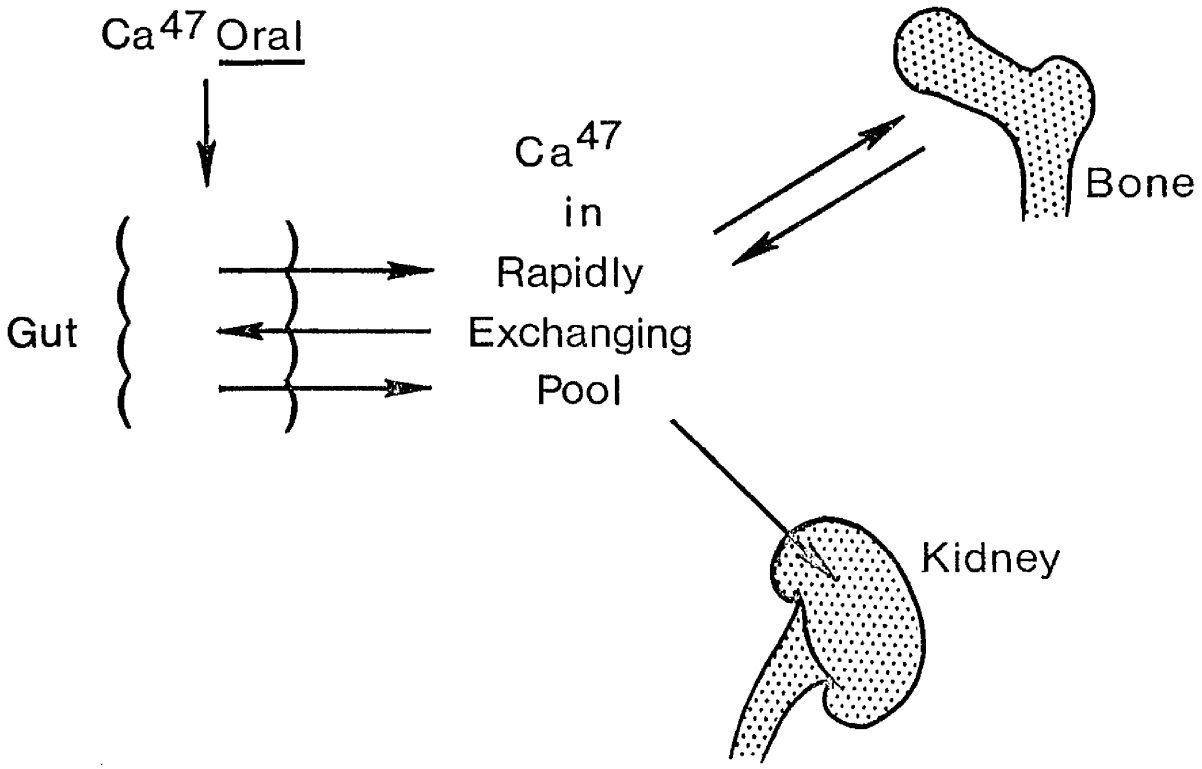


Fig 40 Diagramatic illustration of the pathways followed by an orally administered isotope of calcium (47Ca). The level of the isotope in the blood at time t after administration is a function of the rates of transfer in and out of the rapidly exchanging pool shown in the figure. The isotope is lost permanently from the system by passage down the gut, in the faeces and urinary losses.

CALCIUM ABSORPTION MODEL

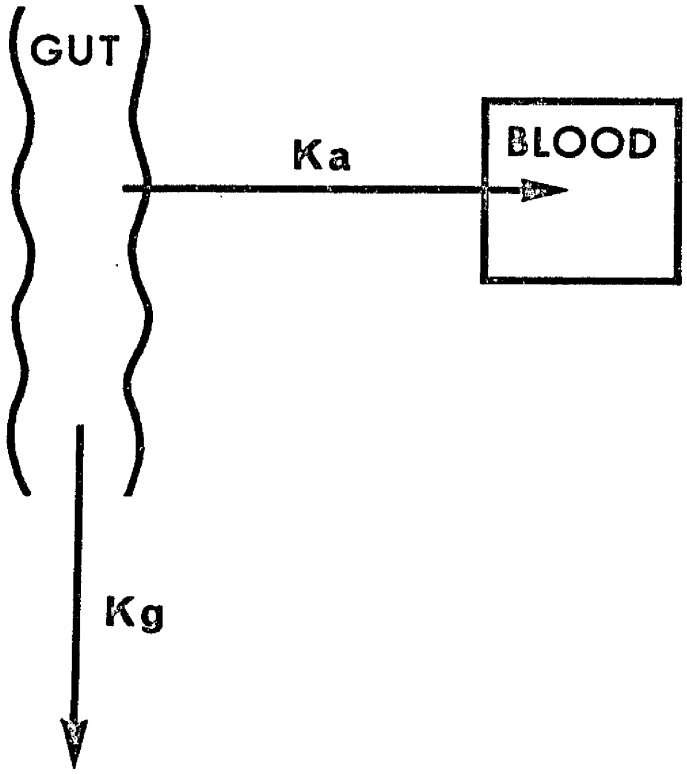


Fig 41 This figure shows the fractional transfer rates of orally administered isotope which have been determined. Ka is the fractional transfer rate from the gut to blood over 6 hours. Kg is the fraction of the isotope which passes down the gut.

CALCIUM ABSORPTION—ANALOGUE MODEL

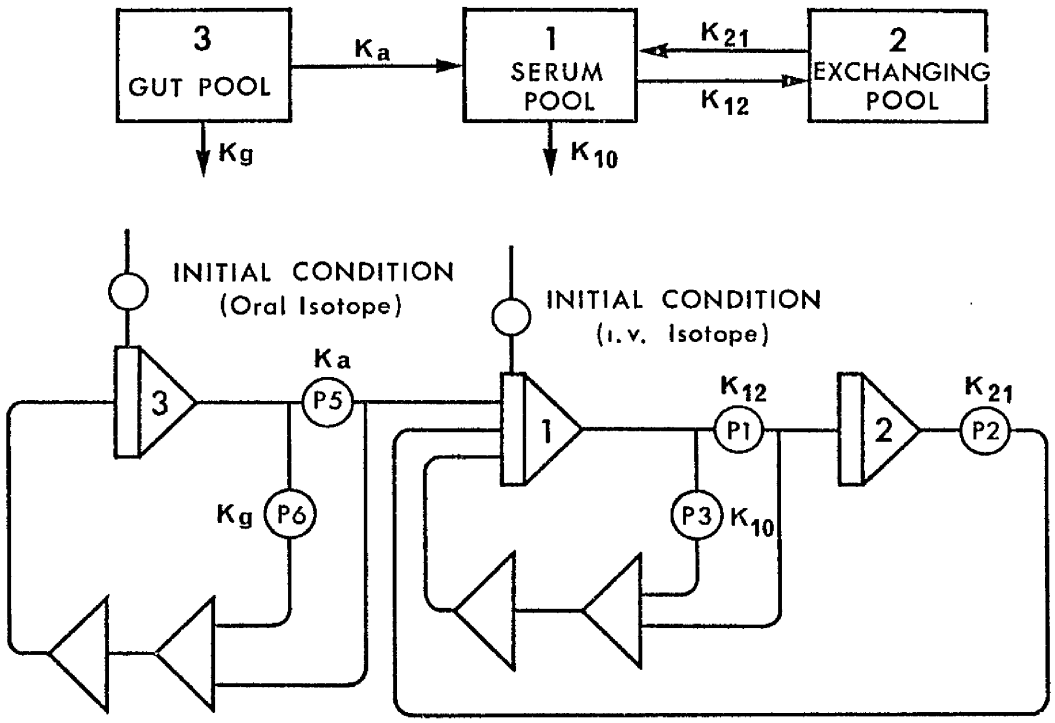


Fig 42 This figure shows the analogue system set up to simulate the change in the intravenously administered isotope, and the transfer of the isotope from the gut to the blood (p. 106).

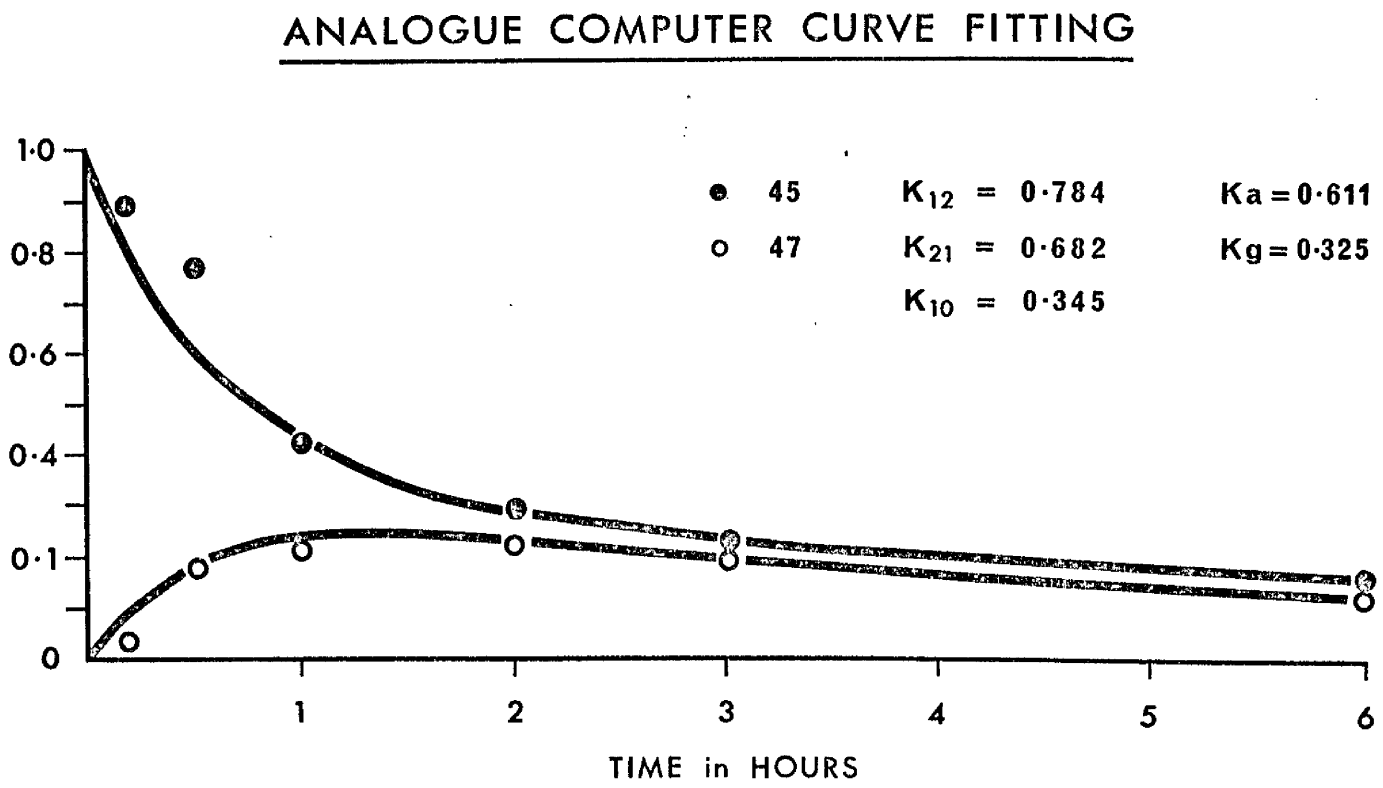


Fig 43 The curves fitted to the data points of the serum specific activities of 45Ca (●) administered intravenously, and oral 47Ca administered simultaneously, against time. Ka is the fractional transfer rate of 47Ca from the gut to blood and Kg the fractional transfer of 47Ca down the gut over the 6 hour period of the study.

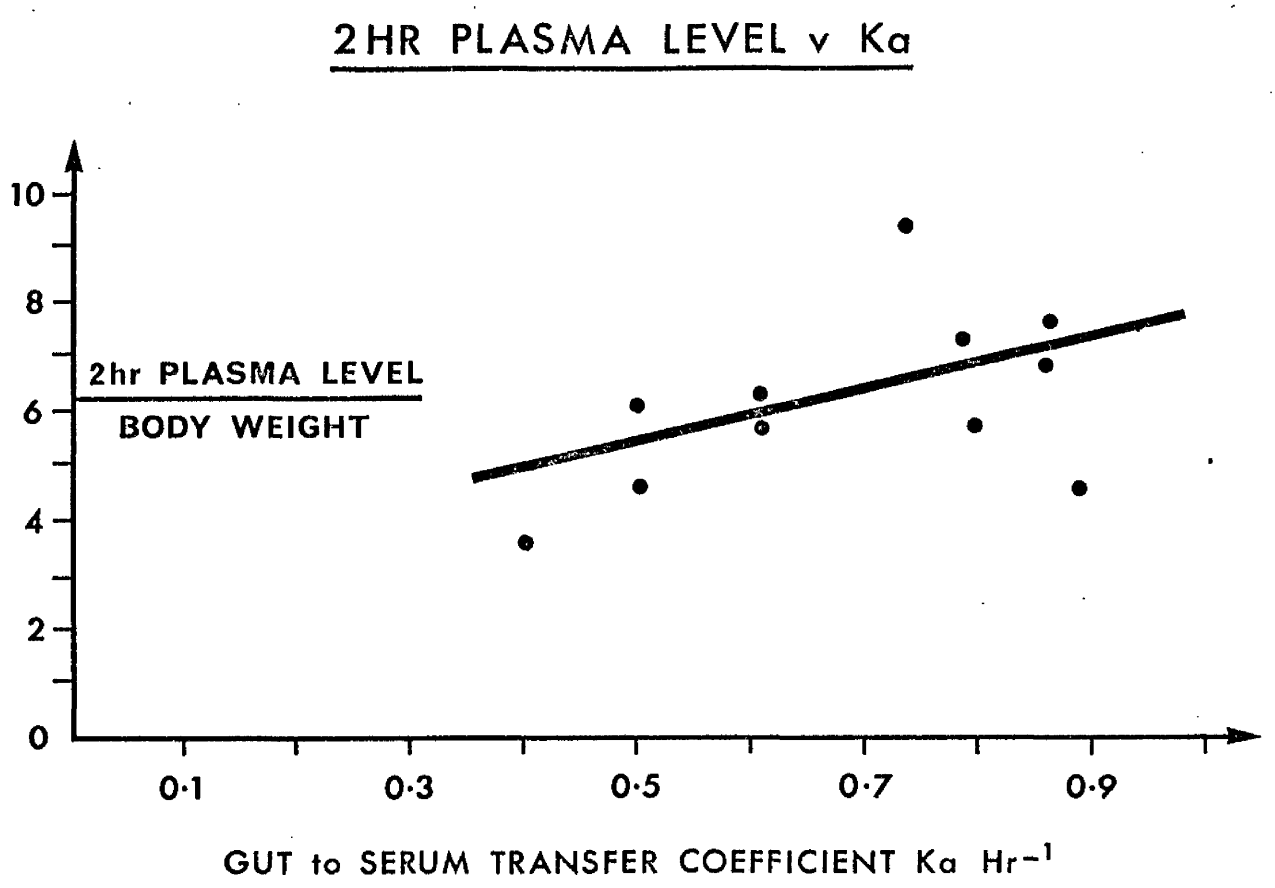


Fig 44 The two hour plasma 47Ca levels multiplied by body weight is significantly related to the fractional transfer rate of the 47Ca from the gut to blood (Ka).

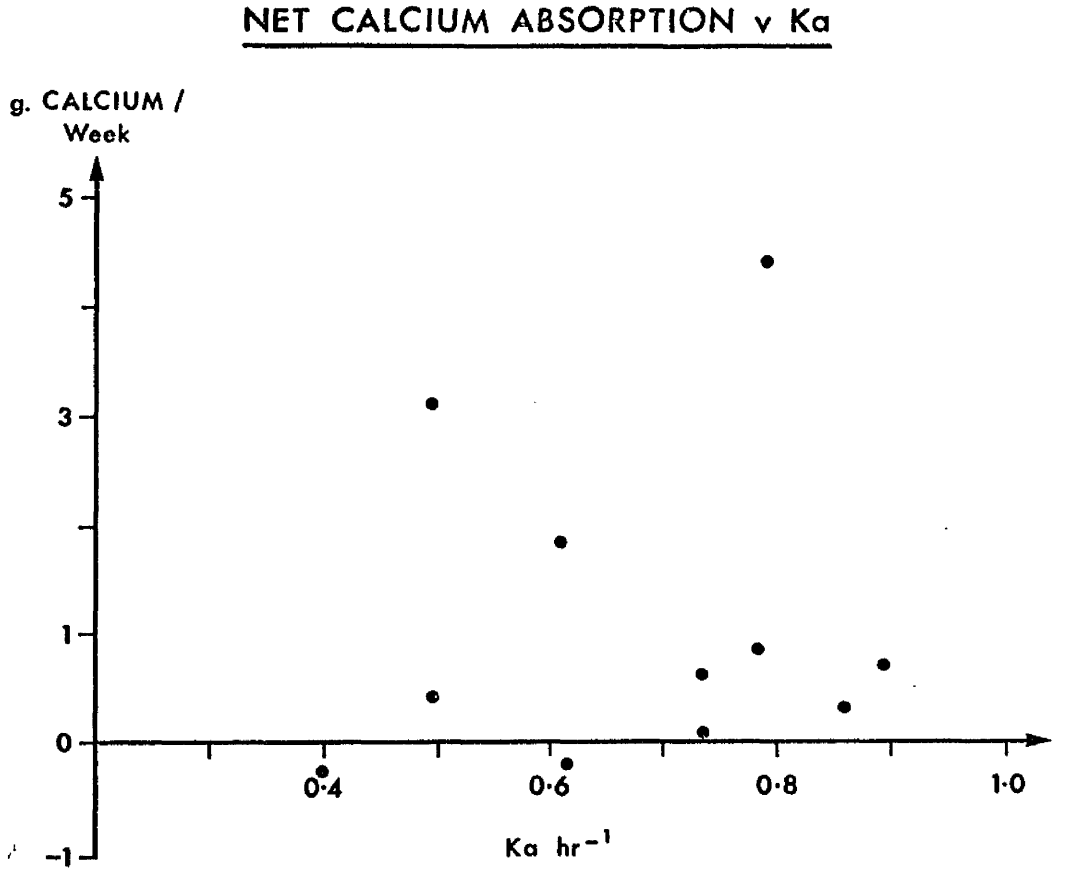


Fig. 45 The net calcium absorption determined from the measurement of calcium balance is not related to the fractional turnover rate from gut to blood estimated by the simultaneous administration of 47Ca orally and 45Ca intravenously.

CHAPTER V

TABLE XIII

Plasma calcium on normal and low calcium diets

Diet	Number of subjects	Number of observations	Mean Plasma Calcium (mg/100 ml)	S.E.
Normal	27	108	9.46*	0.046
Low Calcium	27	108	9.29*	0.043

* Difference between the means = 0.17; t = 2.6; P < 0.01.

TABLE XIV

24-hour urine calcium on normal and low calcium diets

Diet	Number of subjects	Number of observations	Mean 24-hour urine calcium	S.E.
Normal	27	27	198.8*	21.3
Low Calcium	27	27	135.7*	13.8

* Difference between means = 63.1; t = 2.5; P < 0.02.

TABLE XV

Urine calcium:creatinine ratio on normal and low calcium diets

Diet	Number of subjects	Number of observations	Mean urine Ca/Cr	S.E.
Normal	27	27	0.17*	0.016
Low Calcium	27	27	0.12*	0.013

* Difference between means = 0.05; t = 2.5; P < 0.02.

TABLE XVI

The relation between the change in urinary calcium as (i) mg / 24 hrs and (ii) Ca/creatinine ratio and the change in plasma calcium.

	No. of observations	No. of Subjects	Regression equation	r	t	P
(i)	40	39	$y = 143x - 23$	0.46	3.2	<0.01
(ii)	40	39	$y = 14x - 0.025$	0.57	4.3	<0.001

TABLE XVII

The effect of a high phosphorus intake on mean plasma calcium and inorganic phosphorus concentrations in eight subjects with osteoporosis.

Diet	Number of observations	Mean plasma calcium (mg/100 ml)	S.E.	Mean plasma phosphorus (mg/100 ml)	S.E.
Ward	30	9.62	.07	3.51	.18
High phosphorus	29	9.54	.07	3.92	.10
		t = 1.0		t = 2.83	
		P > 0.05		P < 0.01	

TABLE XVIII

The effect of a high phosphorus diet on the urinary phosphate/creatinine ratio, the phosphate/creatinine clearance ratio and the P.E.I. in eight patients with osteoporosis.

Diet	Number of observations	Mean P/cr ratio	S.E.	Mean Cp/Ccr	S.E.	Mean P.E.I.	S.E.
Ward	8	.76	.150	.16	.026	+.03	.022
High phosphorus	8	1.23	.125	.27	.027	+.14	.030
		t = 2.57		t = 3.22		t = 3.12	
		P < 0.05		P < 0.01		P < 0.01	

TABLE XIX

The effect of a calcium infusion on the mean urinary phosphate/creatinine ratio, the phosphate creatinine clearance ratio and the P.E.I. in eight patients with osteoporosis on a high phosphorus diet.

	Number of observations	Mean P/cr ratio	S.E.	Mean Cp/Ccr	S.E.	Mean P.E.I.	S.E.
Before infusion	8	1.48	.207	.31	.025	+.17	.024
After infusion	8	0.65	.210	.11	.025	−.12	.022
		t = 2.99		t = 5.92		t = 9.63	
		P < 0.01		P < 0.001		P < 0.001	

TABLE XX

The effect of a high phosphorus diet on the mean plasma concentrations of calcium and inorganic phosphorus in ten subjects.

Diet	Number of observations	Mean plasma calcium (mg/100 ml)	S.E.	Mean plasma phosphorus (mg/100 ml)	S.E.
Ward	40	9.75	0.071	3.40	0.073
High phosphorus	40	9.51	0.066	4.16	0.105
		t = 2.6		t = 6.08	
		P < 0.01		P < 0.001	

TABLE XXI

Effect of phosphorus supplements on mean concentrations (mg/100 ml) of total and ultrafilterable calcium in nine normal subjects.

	AUTOANALYZER METHOD:				EDTA METHOD:			
	Total calcium		Ultrafilterable calcium		Total calcium		Ultrafilterable calcium	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Controls	9.24	0.05	5.10	0.04	9.67	0.06	5.36	0.07
High P	9.01	0.06	4.81	0.04	9.47	0.11	4.88	0.31
t	3.1		3.1		1.4		2.4	
P	< 0.01		< 0.01		> 0.05		< 0.05	

TABLE XXII

The effect of high phosphorus intake on the calcium/creatinine ratio in eight subjects with osteoporosis. Three observations were made on three successive days before and after starting the high phosphorus intake.

	Calcium/creatinine ratio	
Subject	Ward Diet	High Phosphorus Diet
M.G.	.35	.10
	.15	.13
	.36	.12
J.L.	.28	.17
	.40	.25
	.34	.23
H.L.	.35	.15
	.24	.17
	.26	.18
R.B.	.20	.19
	.19	.22
	.39	.12
I.B.	.29	.14
	.37	.13
	.12	.16
H.W.	.42	.32
	.44	.26
	.37	.30
A.M.	.20	.08
	.15	.13
	.11	.09
M.S.	.29	.33
	.28	.15
	.24	.23
Number of observations	24	24
Mean Ca/Cr	.283	.180

$t = 4.23$

$P < 0.001$

TABLE XXIII

Age, sex, weight and creatinine clearance in 8 "osteoporotic" subjects, 9 patients with renal stone, 8 patients with hyperparathyroidism, and 2 patients with hypoparathyroidism

Diagnostic group	Patient	Age	Sex	Weight (Kg)	Creatinine Clearance (ml/min)
Osteoporosis	R.B.	60	F	61	119
	C.H.	36	F	45	125
	E.T.	61	F	59	88
	J.M.	57	M	57	141
	A.M.	54	M	55	97
	E.B.	61	M	50	97
	D.B.	55	F	68	101
	A.S.	67	M	60	98
Renal stone	A.C.	51	F	58	100
	W.H.	55	M	65	102
	J.H.	51	M	71	120
	M.S.	68	F	55	110
	J.Q.	33	M	67	140
	M.M.	53	F	60	80
	A.D.	20	M	75	137
	T.B.	37	M	81.5	134
	S.B.	40	F	64	73
Hyperparathyroidism	H.C.	46	F	55	97
	F.B.	38	M	61	125
	J.R.	45	M	58	83
	S.M.	48	F	49	94
	W.C.	47	F	64	78
	D.S.	40	M	63	88
	E.S.	37	F	51	140
	J.W.	57	M	71	110
Hypoparathyroidism	S.G.	20	F	45	89
	E.B.	43	F	79	102

TABLE XXIV

Whole bone density and percentile values
in 8 "osteoporotic" subjects

Patient	Age	Sex	SAE	Percentile
R.B.	60	F	27.5	38
C.H.	36	F	32	19
E.T.	61	F	26.6	34
J.McG.	57	M	31.5	48
A.M.	54	M	27	13
E.B.	61	M	28	27
D.B.	55	F	26	15
W.N.	67	M	25	27

TABLE XXV

The relation between the calcium/creatinine clearance ratio and the plasma ultrafilterable calcium in osteoporosis, renal stones, hyperparathyroidism and hypoparathyroidism.

Diagnostic group	Regression equation	r	P
Oosteoporosis	$y = 0,016x - 0,019 \pm 0,068$	0,47	< 0,0001
Renal stones	$y = 0,049x - 0,167 \pm 0,12$	0,63	< 0,001
Hyperparathyroidism	$y = 0,018x - 0,065 \pm 0,050$	0,66	< 0,001
Hypoparathyroidism	$y = 0,052x - 0,215 \pm 0,10$	0,87	< 0,001

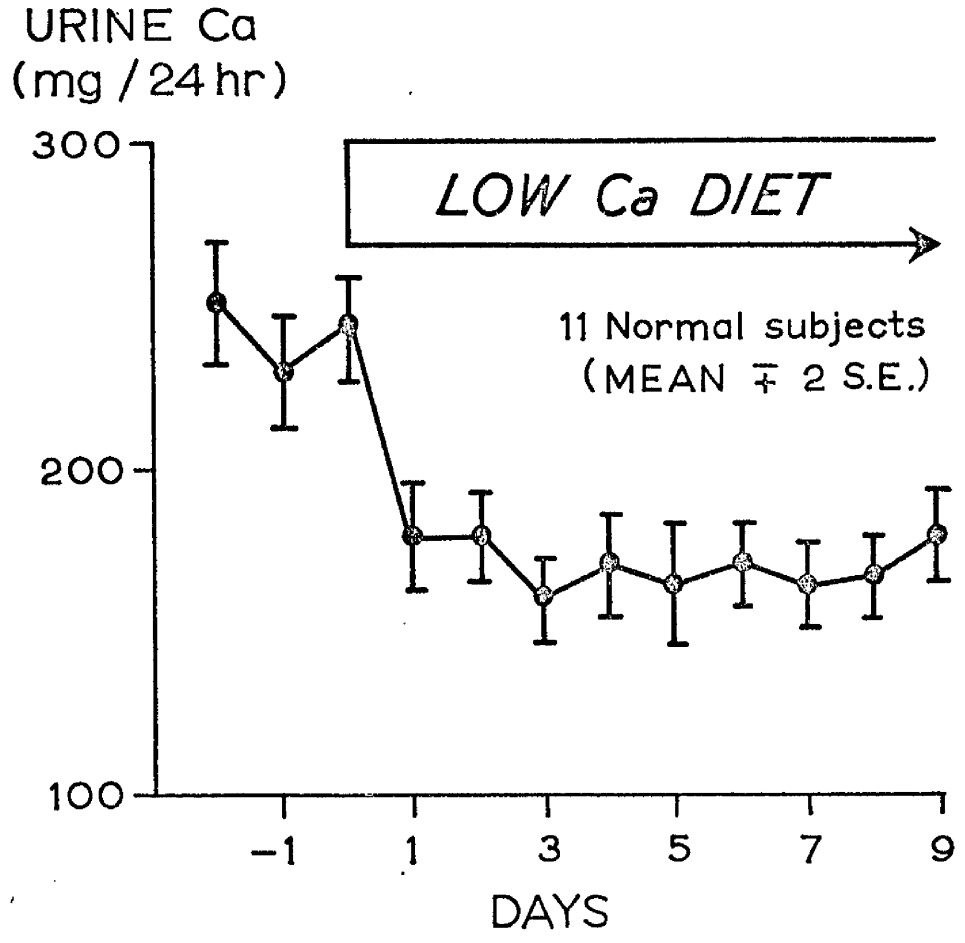


Fig 46 The fall in the 24 hour urine calcium output in normal subjects when changed from a free to a low calcium diet. The significant fall in urinary calcium excretion is apparent within 24 hours and no significant fall is apparent thereafter. The mean and 2 S.E. range of urinary calcium excretion is shown.

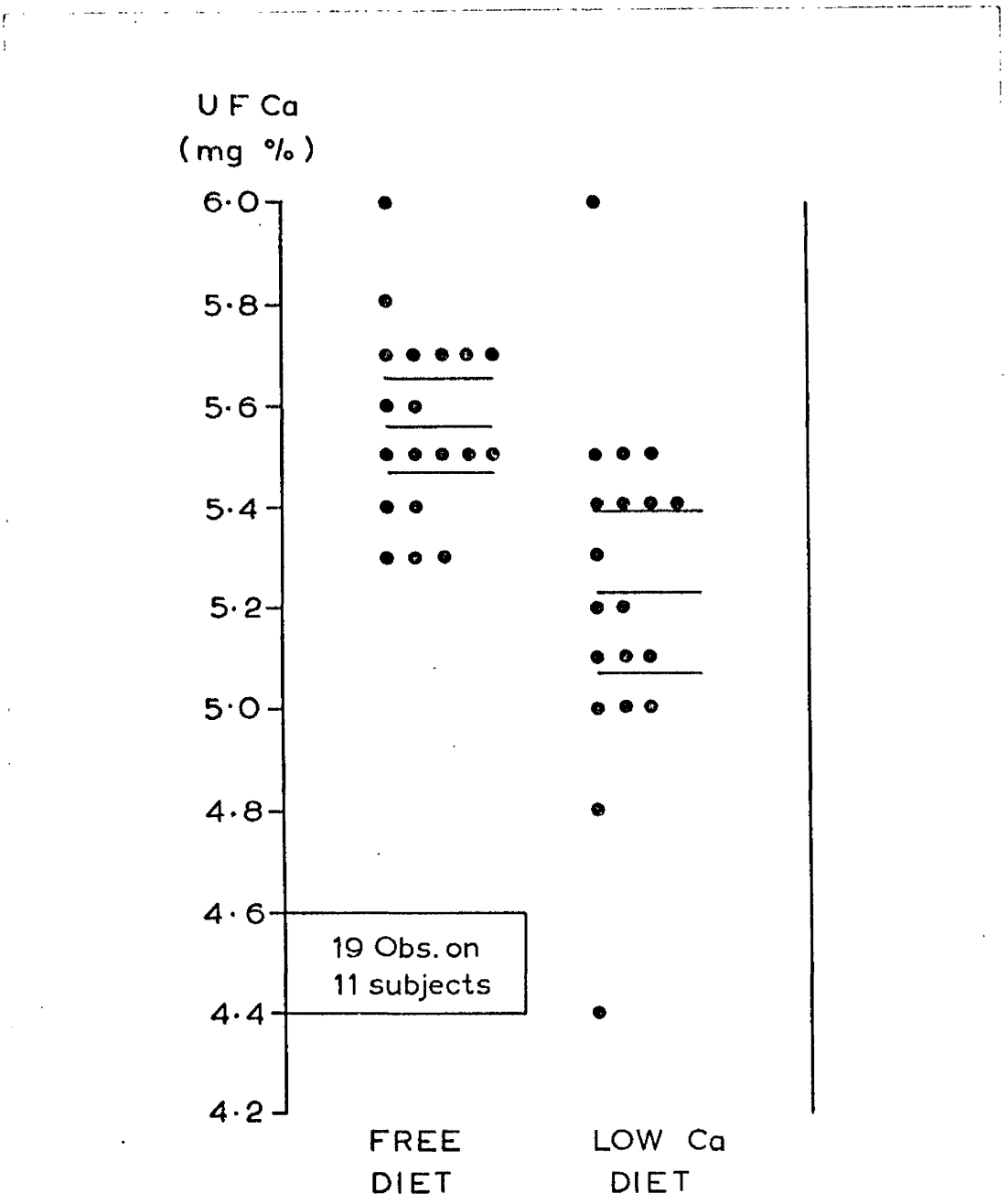


Fig 47 The ultrafilterable calcium in 11 normal subjects on a free and low calcium diet. The fall in ultrafilterable calcium on a low calcium diet is highly significant ($P < 0.001$).

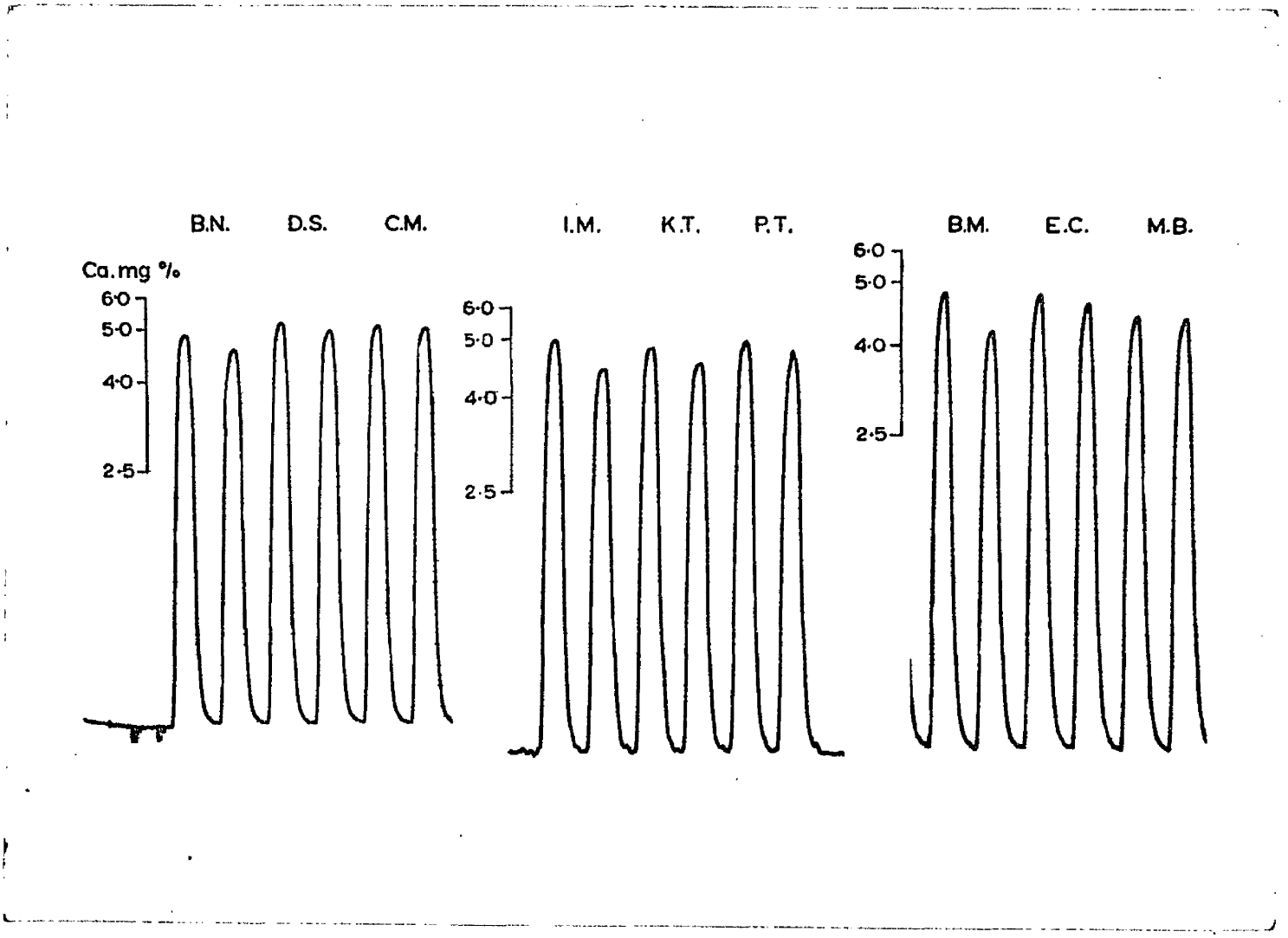


Fig 48 AutoAnalyzer tracing of the calcium concentration on a free and a low calcium diet. The serum samples of the subjects on the two diets were fed in alternately and the difference between the two values is apparent from the differences in peak height.

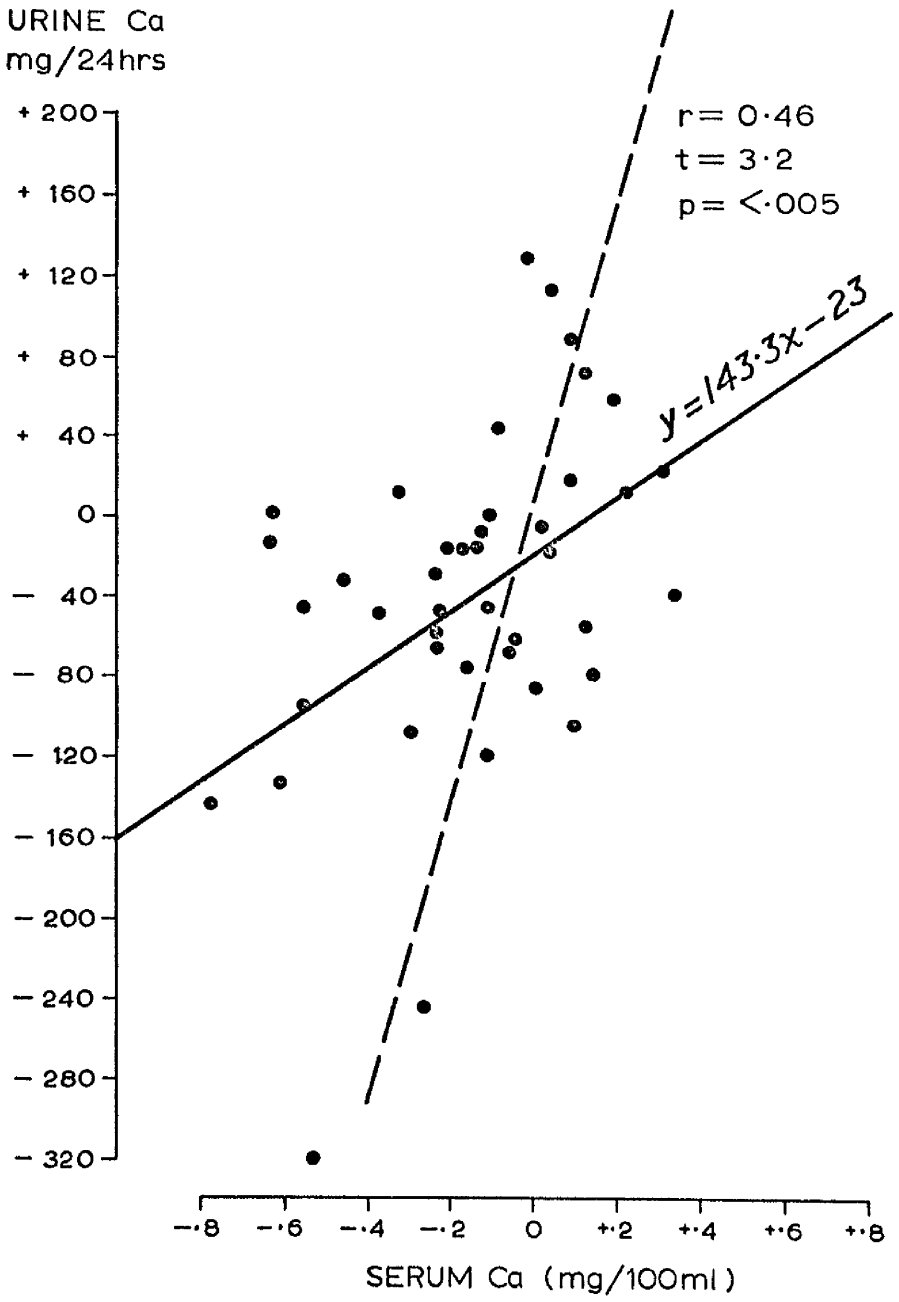


Fig 49 The change in 24 hour urinary calcium excretion is significantly related to the change in plasma calcium in patients on low and high calcium diets. The continuous line shows the relation between the two values. The interrupted line shows the change that would be expected if the tubular resorption of calcium had remained constant.

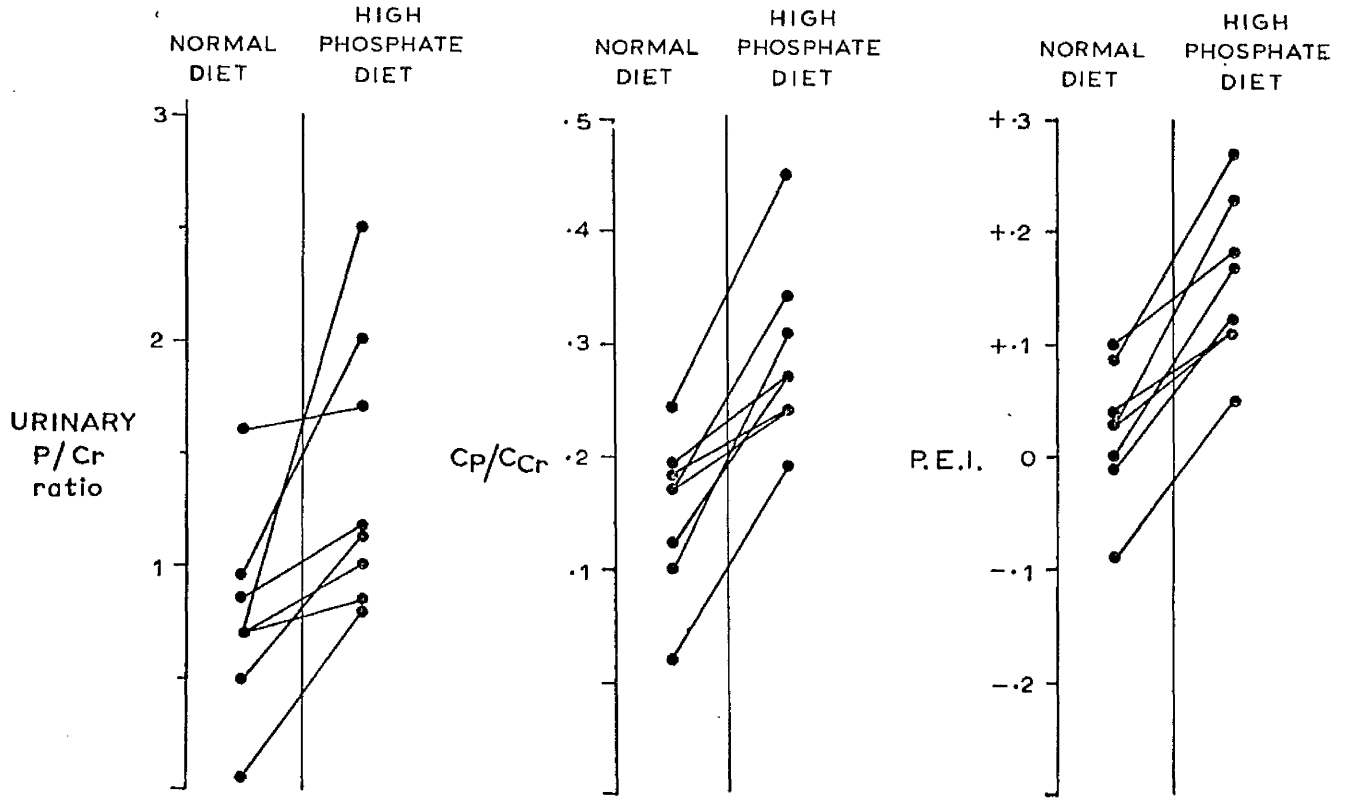


Fig 50 The effect of high phosphate feeding on the urinary phosphate/creatinine ratio, the phosphate/creatinine clearance ratio and the Phosphate Excretion Index.

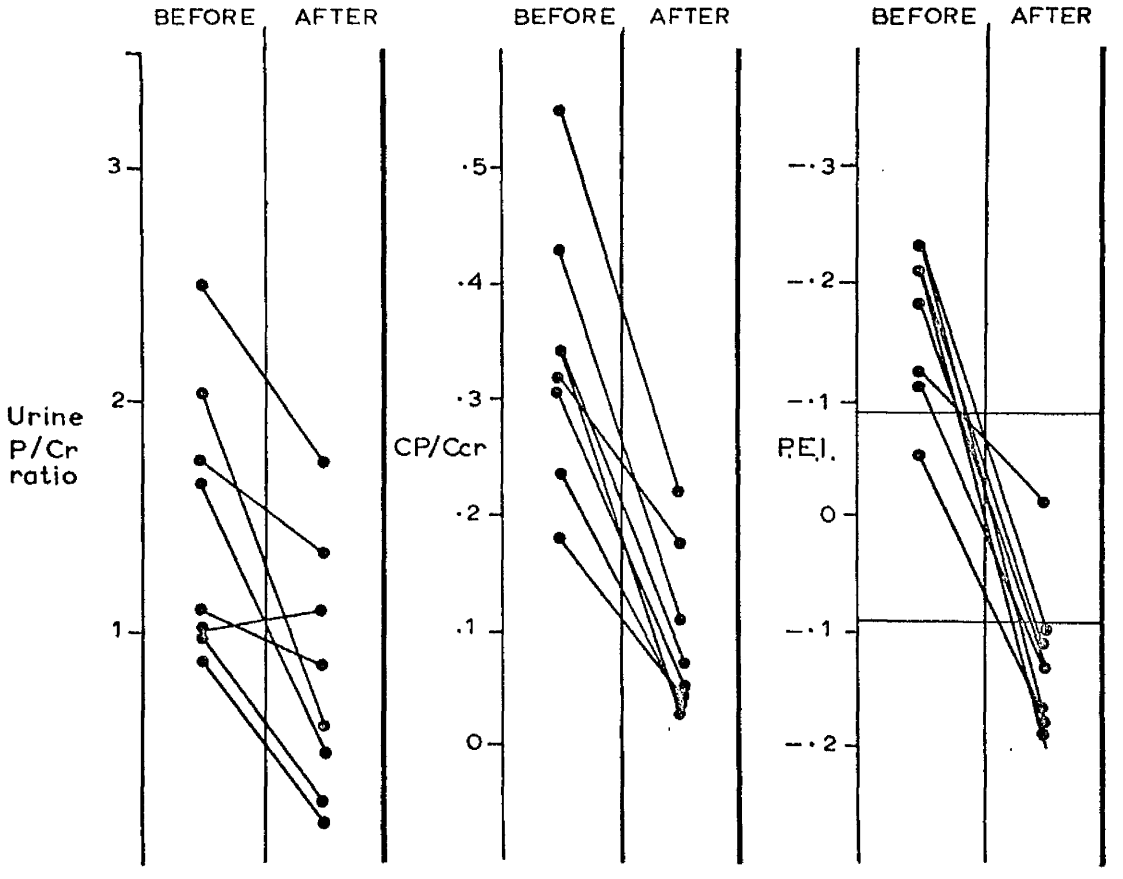


Fig 51 The effect of calcium infusions on the ratio of phosphate to creatinine in the urine, on the phosphate/creatinine clearance ratio and the Phosphate Excretion Index, in patients on high phosphate feeding.

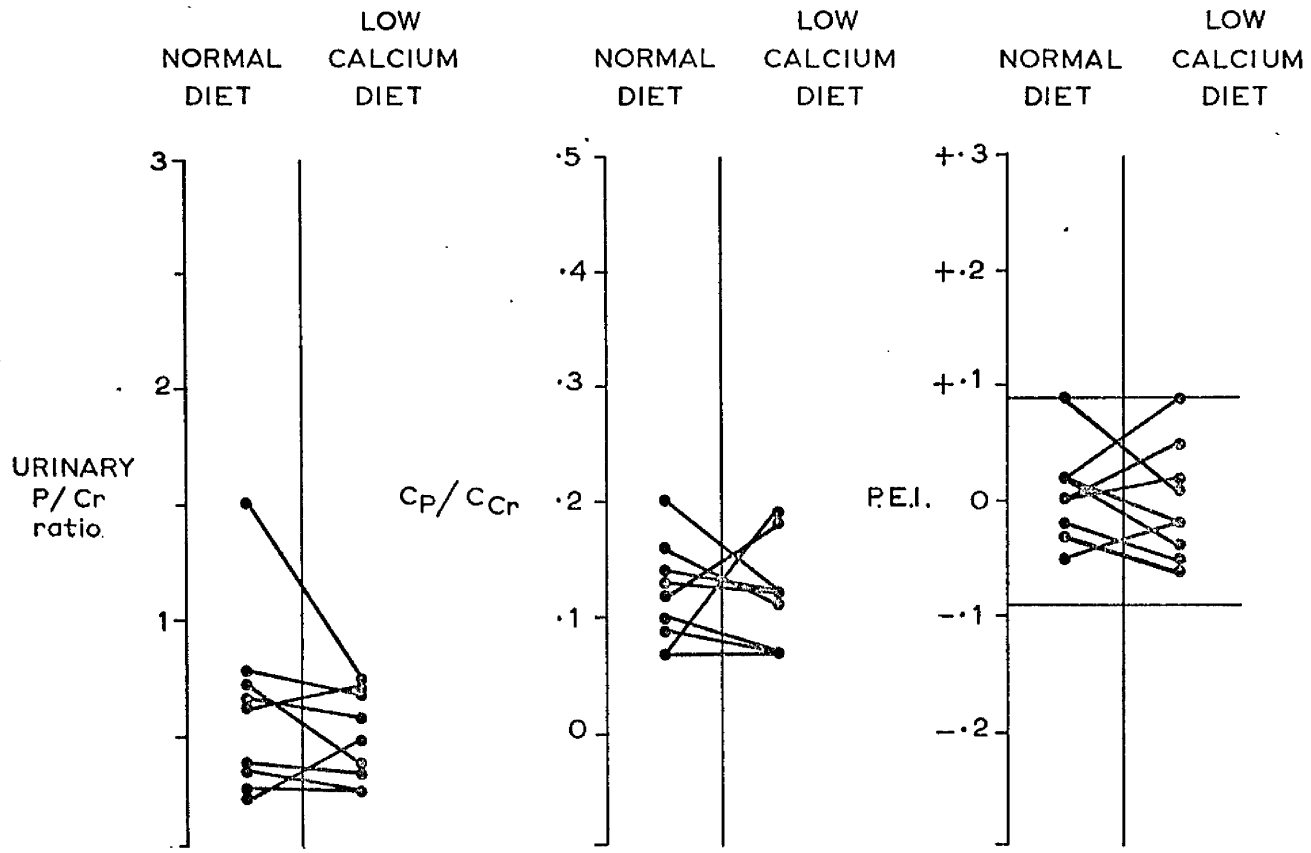


Fig 52 The urinary phosphate/creatinine ratio, phosphate/creatinine clearance ratio and Phosphate Excretion Index in patients on normal and low calcium diet. No significant effect on phosphate excretion is evident.

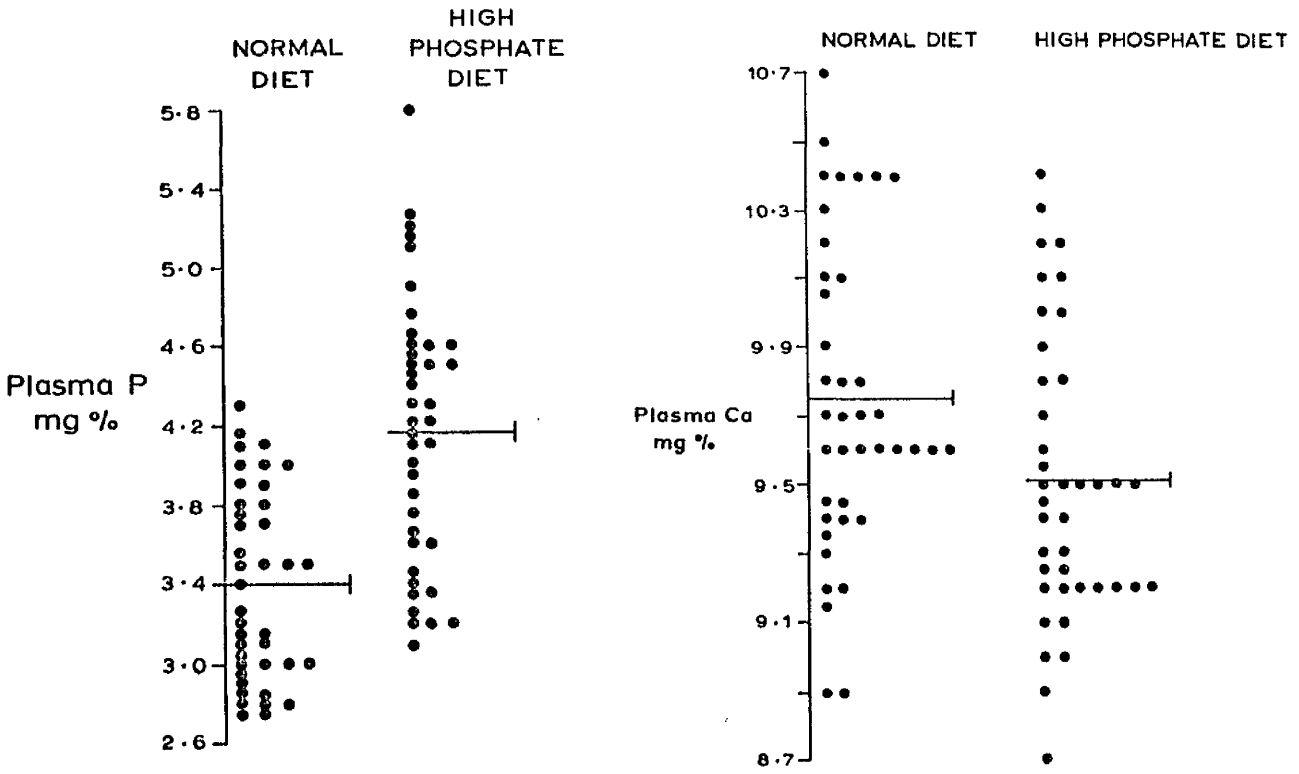


Fig 53 Changes in plasma phosphate and plasma calcium in patients on a normal and a high phosphate diet. There is a significant rise in plasma phosphorus and a fall in plasma calcium.

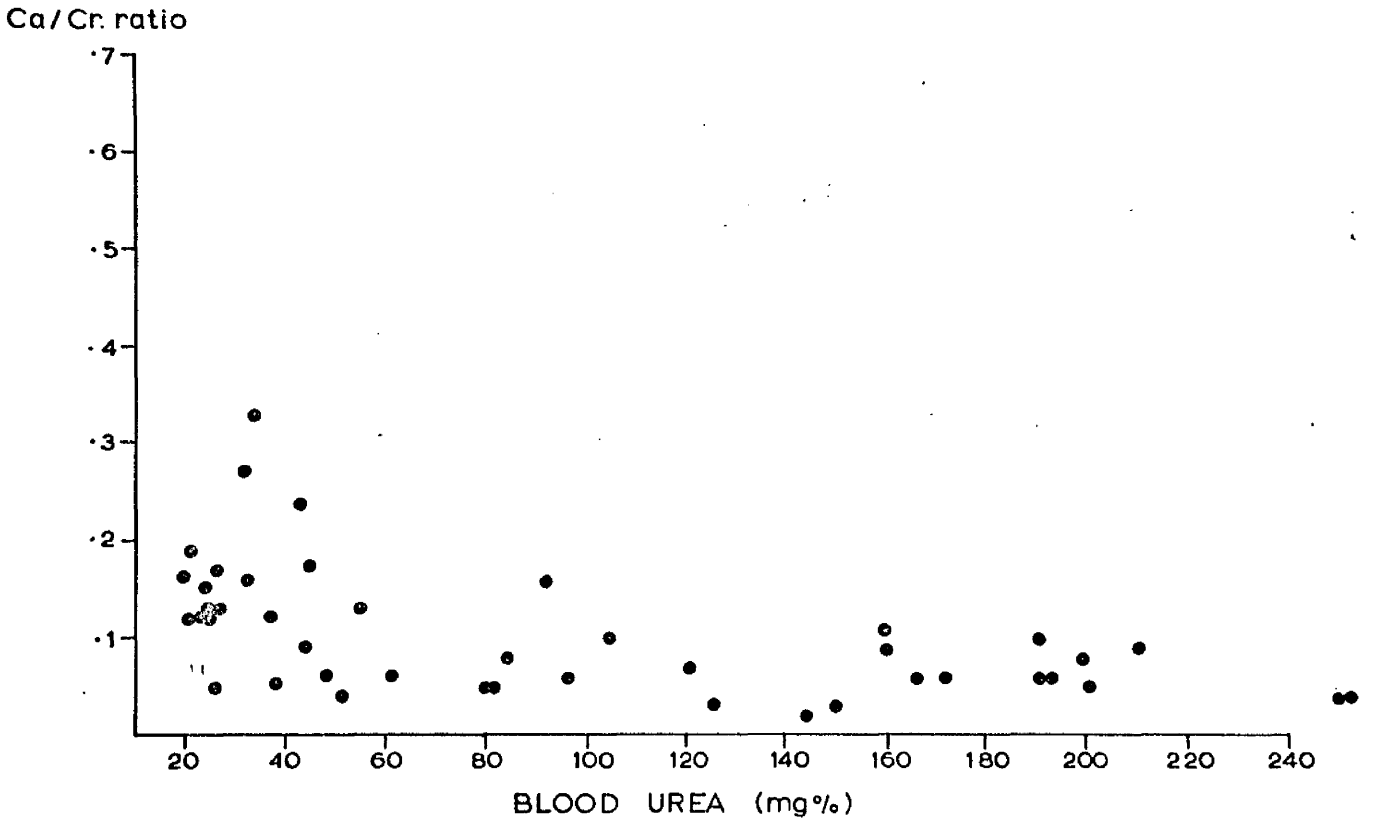


Fig 54 The change in calcium/creatinine ratio with renal failure.

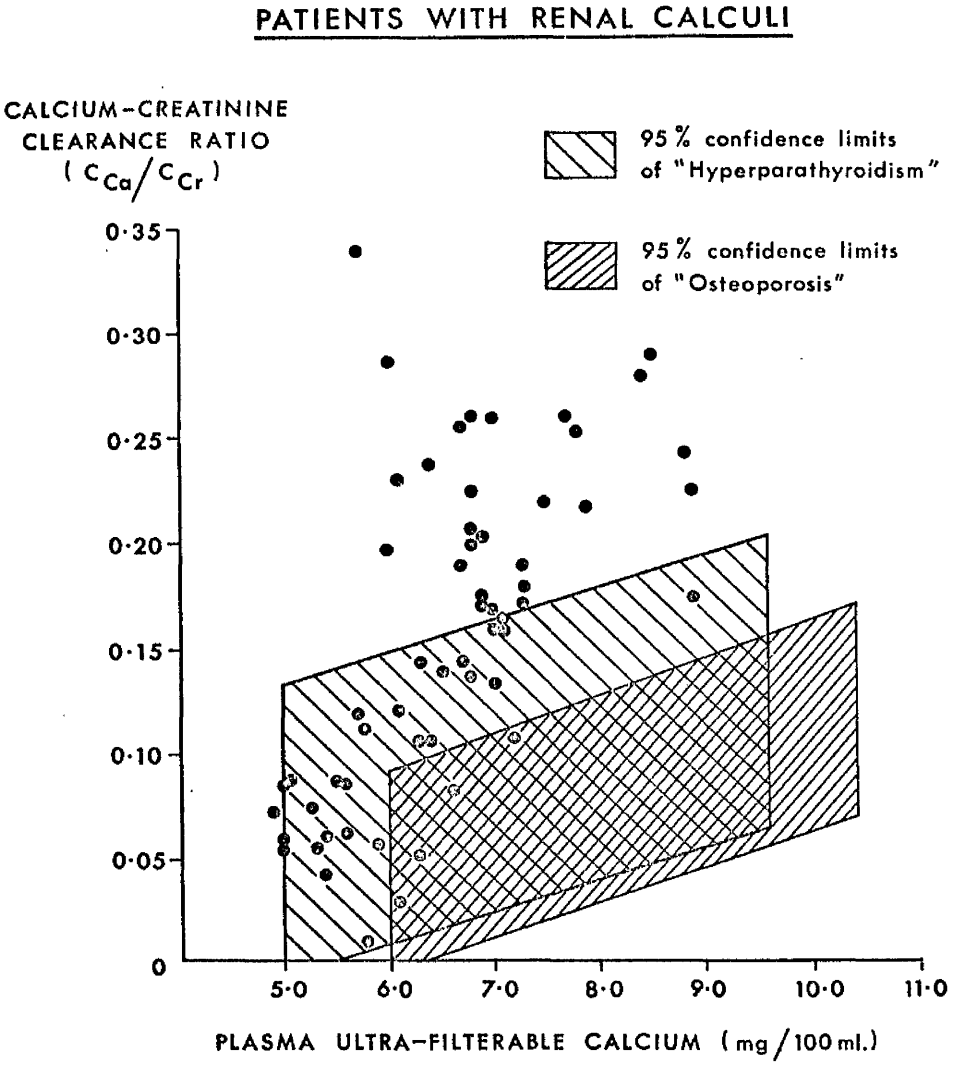


Fig 55 This figure shows the relation of the calcium/creatinine clearance ratio to plasma ultrafilterable calcium in patients with "osteoporosis", primary hyperparathyroidism and renal stone disease (•).

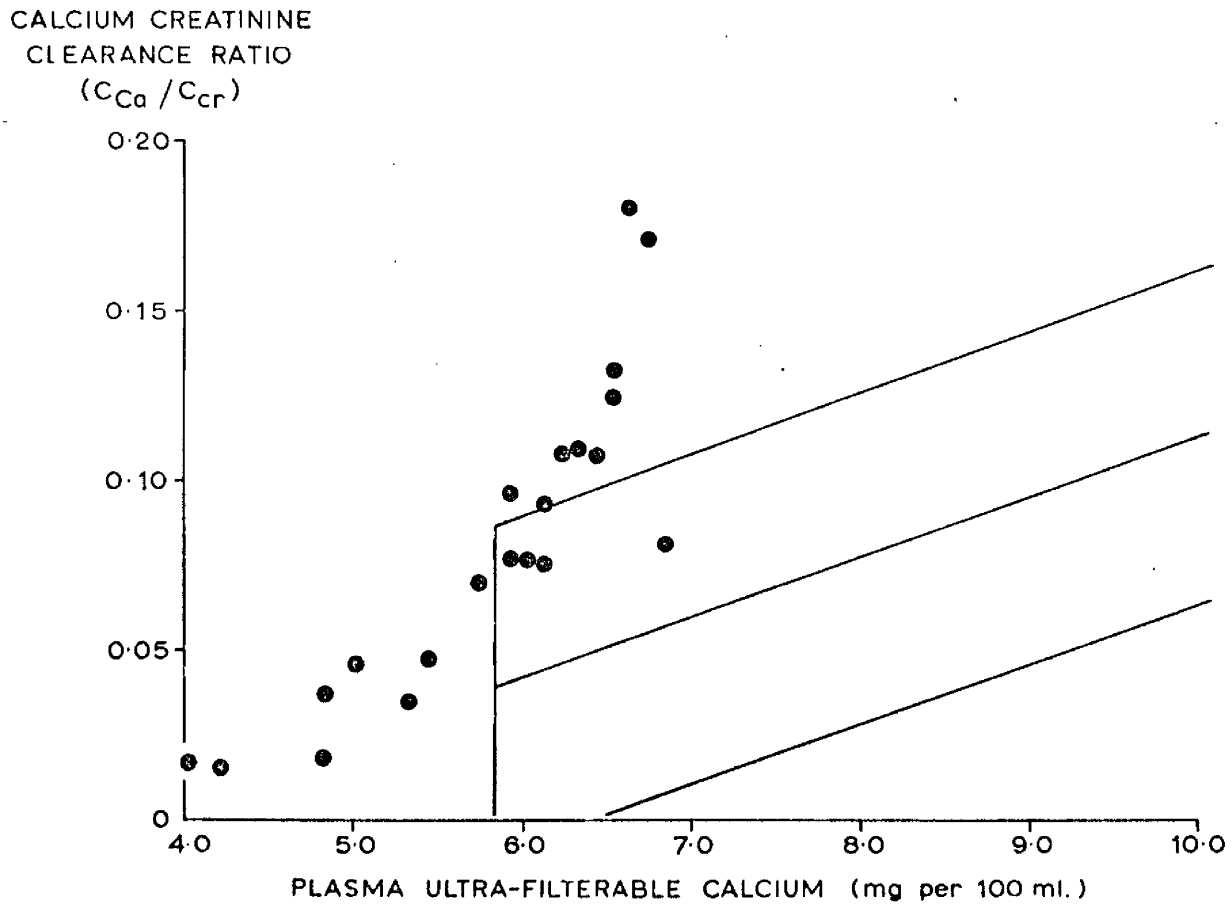


Fig 56 The relation between the calcium/creatinine clearance ratio and the plasma ultrafilterable calcium in patients with hyperparathyroidism and patients with hypoparathyroidism (o). The 95% Confidence limit for the osteoporotic subjects is shown.

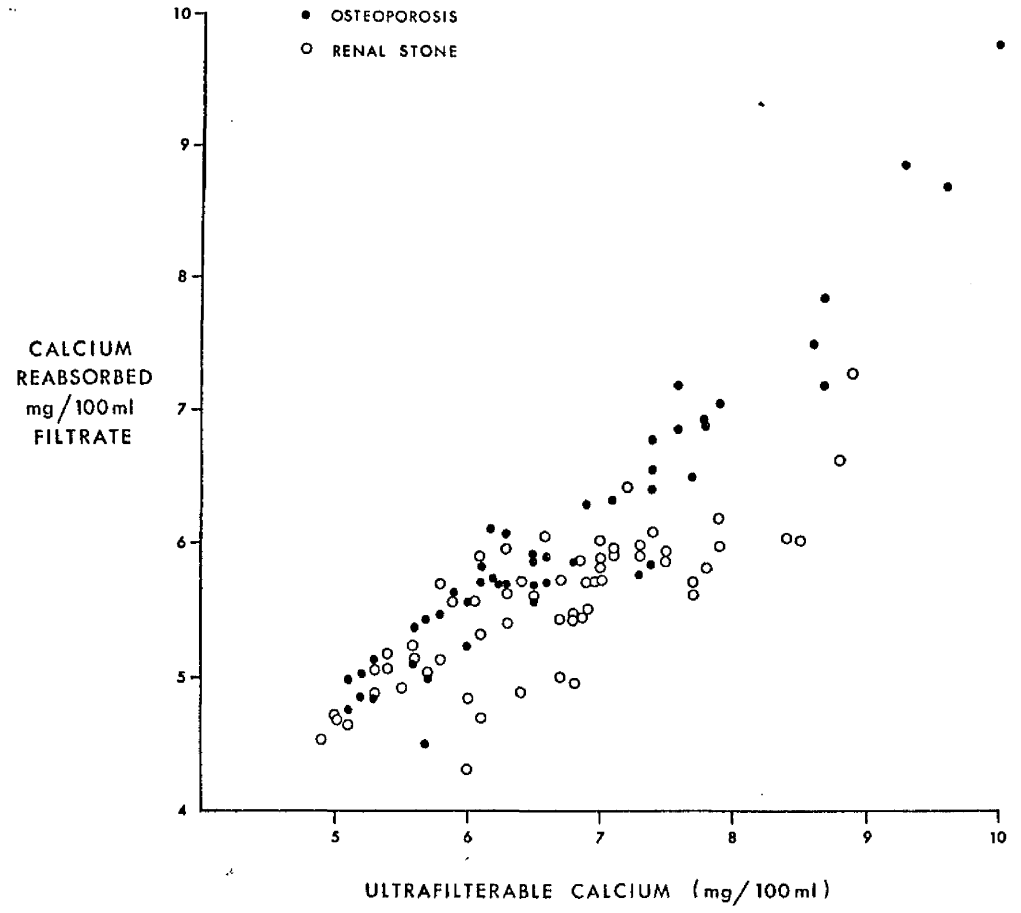


Fig 57 The relation between the calcium reabsorbed per 100 ml of glomerular filtrate and the plasma ultrafilterable calcium in patients with "osteoporosis" and patients with renal stone disease.

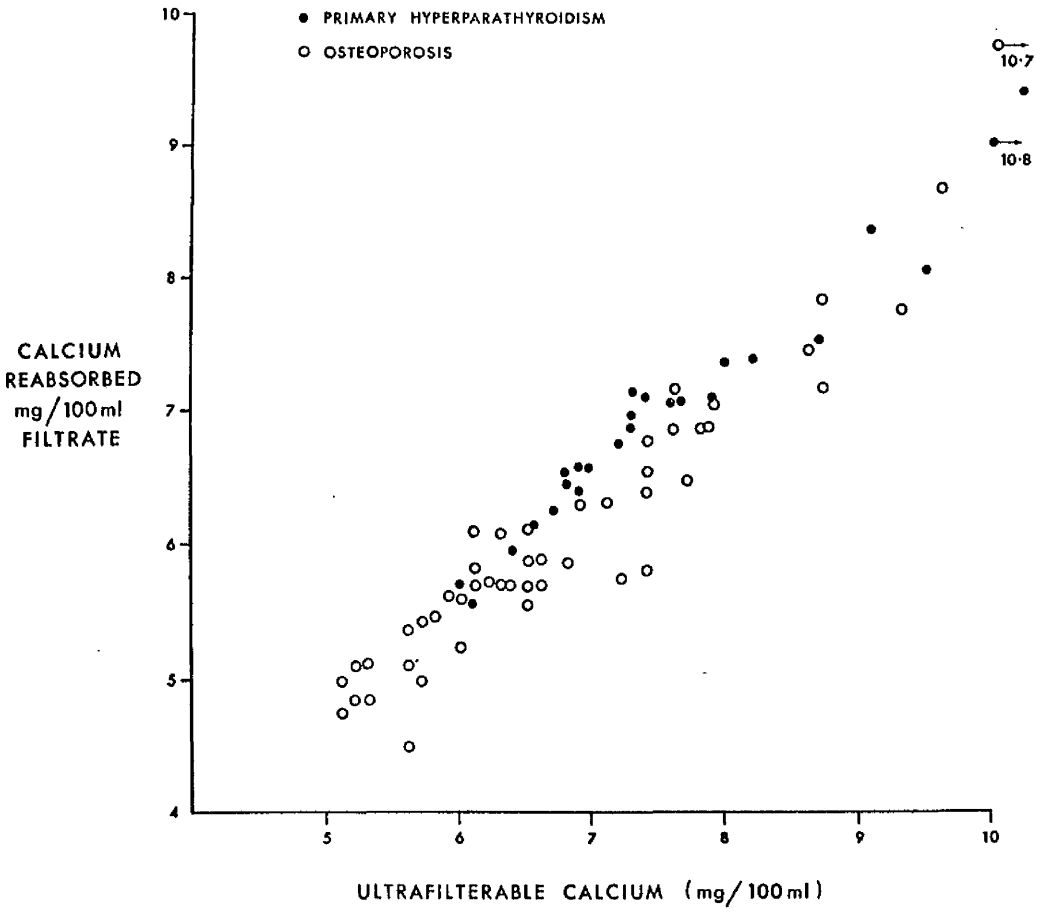


Fig 58 The relation between the calcium reabsorbed per 100 ml of glomerular filtrate and the plasma ultrafilterable calcium in patients with "osteoporosis" and patients with primary hyperparathyroidism. The mean calcium resorption per 100 ml of filtrate is significantly higher in the hyperparathyroid patients between 6 and 9 mg% of ultrafilterable calcium.

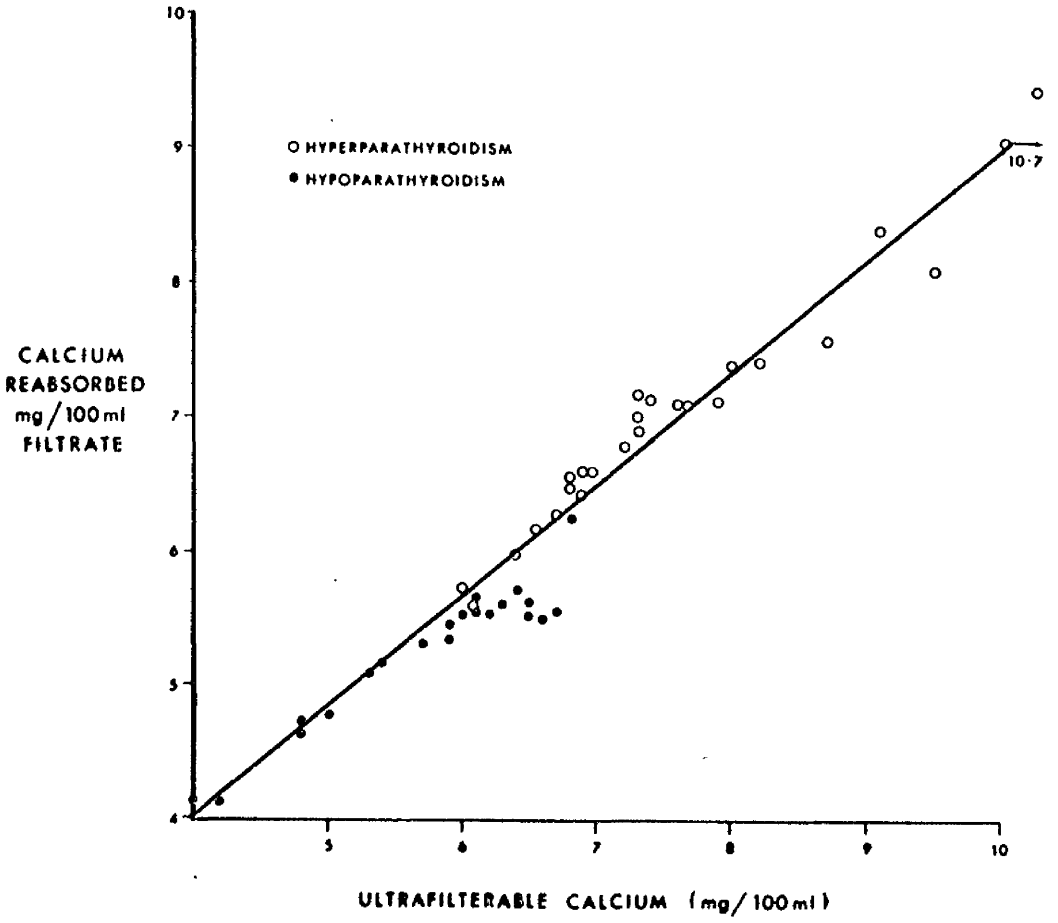


Fig 59 The calcium reabsorbed per 100 ml of filtrate plotted against the plasma ultrafilterable calcium in patients with hyperparathyroidism and in patients with hypoparathyroidism. The fall in the reabsorption of calcium is evident in the hypoparathyroid patients above 5.5 mg% plasma ultrafilterable calcium.

CHAPTER VI

TABLE XXVI

Clinical details of patients given isotopes by continuous feeding

Case	Age	Sex	Weight (Kg)	Diagnosis	Bone status
Patients given 45Ca and 85Sr:					
P.C.	55	M	48	Steatorrhoea	Osteoporosis
J.M.	59	M	52	Diarrhoea	Normal
R.T.	50	M	72	Osteoporosis	Osteoporosis
A.D.	49	M	52	Steatorrhoea	Normal
J.L.	76	F	37	Osteoporosis	Osteoporosis
T.R.	64	M	51	Osteoporosis	Osteoporosis
F.A.	60	F	57	Osteoporosis	Osteoporosis
R.W.	63	M	57	Rheumatoid arthritis (steroids)	Osteoporosis
J.L.	63	F	66	Backache	Normal
A.M.	54	M	56	Osteoporosis	Osteoporosis
F.D.	26	M	56	Osteoporosis	Osteoporosis
J.P.	59	F	50	Rheumatoid arthritis	Osteoporosis
T.C.	48	M	45	Steatorrhoea	Osteomalacia
Patients given 45Ca:					
H.L.	65	F	67	Osteoporosis	Osteoporosis
M.S.	53	F	43	Rheumatoid arthritis	Rheumatoid arthritis (steroids)
I.K.	43	F	62	Osteoporosis	Osteoporosis
A.S.	46	F	62	Osteoporosis	Osteoporosis
A.H.	67	F	38	Steatorrhoea	Steatorrhoea
F.S.	70	F	46	Renal failure	Renal failure
Patients given 85Sr:					
A.McS.	50	M	47	Steatorrhoea	Osteoporosis
M.G.	43	F	90	Arthritis	Normal
J.McN.	58	M	80	Myocard. infarc.	Normal
A.M.	80	F	52	Osteoporosis	Osteoporosis
M.R.	57	F	58	Osteoporosis	Osteoporosis
M.H.	58	F	41	Gastroenteritis	Osteoporosis
J.I.	68	F	61	Osteoporosis	Osteoporosis
E.B.	60	F	35	Steatorrhoea	Osteomalacia
F.M.	60	F	58	Osteoporosis	Osteoporosis
M.McM.	57	F	46	Osteoporosis	Osteoporosis
M.McE.	72	F	43	Osteoporosis	Osteoporosis
A.McL.	78	F	48	Osteoporosis	Osteoporosis
D.V.	68	F	61	Osteoporosis	Osteoporosis
M.McL.	72	F	61	Osteoporosis	Osteoporosis
W.Y.	60	F	57	Osteoporosis	Osteoporosis
E.W.	75	F	40	Renal failure	Osteomalacia
C.W.	31	M	82	Calculi	Normal
E.S.	59	F	69	Calculus	Osteoporosis
K.C.	34	M	70	Hypertension	Normal

TABLE XXVII

Bone-mineralization rates (mg/Kg/day) measured by continuous feeding of strontium-85 and calcium-45.

Case	Bone-mineralization rate measured by ^{85}Sr	Bone-mineralization rate measured by ^{45}Ca
P.C.	5.0	9.7
J.M.	4.8	10.5
R.T.	0	7.0
A.P.	13.4	13.0
T.R.	33.0	8.0
T.R.	22.0	8.7
F.A.	18.5	7.2
F.A.	12.4	14.0
R.W.	8.3	4.6
R.W.	8.8	3.9
R.W.	7.9	5.3
J.L.	5.5	4.9
J.L.	0.9	7.2
J.L.	6.6	7.3
A.M.	12.5	7.4
A.M.	3.2	8.4
A.M.	10.5	10.2
F.D.	15.6	4.5
F.D.	22.0	8.0
J.P.	21.0	9.5
J.P.	11.6	8.7
T.C.	13.0	10.0
Mean	11.7	8.1
Standard error	1.7	0.6

TABLE XXVIII

Patients given intravenous calcium-45 and strontium-85
M values are expressed in mg/day

Case	Age	Weight (Kg)	Diagnosis	85SrM	45CaM
J.R.	64	50	Osteoporosis	220	234
H.G.	53	72	Osteoporosis	176	306
A.P.	70	44	Osteoporosis	60	358
F.T.	62	48	Renal failure	103	141
M.McK	69	46	Osteoporosis	138	153
E.T.	64	54	Osteoporosis	170	181

TABLE XXIX

Comparison of 85Sr retention measured by the whole body counter, and isotope excretion in Urine and faeces in 3 osteoporotic patients.
(W.B.C. is Percent retention estimated on the whole body counter)

Patient (a) Age 67 yrs			Patient (b) Age 64 yrs			Patient (c) Age 65 yrs		
	% Retention			% Retention			% Retention	
Day	W.B.C.	Excretion	Day	W.B.C.	Excretion	Day	W.B.C.	Excretion
0	100	100	0	100	100	0	100	100
7	49.4	49.6	5	42	42.1	8	29.6	30.6
14	36.7	33.1				15	22.7	23.4
21	31.1	29.0				22	20.1	20.9

TABLE XXX

Mineral Transfer Rate (M.T.R.) measured by the method of Bauer et al (1957) between 7 and 14 days after injection of the isotope.

Patient	Age	Weight (Kg)	M.T.R. (mg Ca/day)	Metacarpal Index
M.M.	59	53	584	43
E.B.	72	42	261	31
M.J.	64	60	405	28
F.H.	70	50	273	49
E.H.	64	51	135	41
I.W.	46	74	435	39
J.W.	56	43	361	48
C.W.	58	64	134	53
H.M.	82	69	182	48
E.McS.	75	67	266	44
E.McK.	66	57	281	43
M.McK.	75	-	192	-
M.McL.	70	59	277	45
J.McL.	79	46	196	21
M.H.	85	70	206	42
A.H.	61	61	301	37
H.G.	80	57	288	54
I.G.	63	65	211	37
M.G.	31	53	226	48
M.G.	58	69	245	-
E.B.	66	66	305	59
M.L.	63	68	303	47
M.K.	81	63	111	37
M.K.	65	57	241	49
J.L.	63	65	280	59
M.M.	30	53	227	57
A.M.	80	53	103	46
M.M.	63	44	166	40
M.McI.	84	69	187	44
J. McH.	54	69	79	71
E. McI.	85	63	83	43
M. McE.	67	44	117	33
R.B.	60	61	86	57
H.G.	69	58	122	51

Cont'd../

TABLE XXX Contd.

Patient	Age	Weight (Kg)	M.T.R. (mg Ca/day)	Metacarpal Index
C.C.	68	61	131	40
A.H.	78	53	142	36
L.G.	67	60	198	58
J.B.	51	58	198	69
A.Y.	80	55	188	43
M.M	55	63	195	49
E.T.	54	53	196	50
E.L.	75	54	281	43
V.M	68	43	287	43
E.L.	80	61	87	47
J.C.	72	53	39	36
J.C.	58	69	228	54
B.F.	58	69	392	35
E.D.	70	70	39	70
M.S.	52	70	338	50
M.S.	71	-	325	47
A.S.	48	57	254	58
B.L.	95	48	124	37
G.M.	75	44	153	47
I.M.	42	65	356	61
N.N.	57	56	553	56
S.O.	74	-	155	45
J.S.	77	68	242	31
J.R.	64	61	220	48
H.G.	53	58	176	49
A.P.	70	54	60	40
F.T.	62	56	103	46
M.McK.	69	46	138	50
E.T.	64	51	170	31
E.E.	69	39	220	38
E.McK.	59	40	216	43

Summary of all data broken down into separate studies according to the ^{40}Ca intake.
Each value is the mean for the number of weeks shown in column 1.

Case	Age	Sex	Wt. Kg.	Diagnosis	Bone status	1 Duration of study+	2 Intake 40Ca i mg/kg/day	3 S.A. i* %dose/mg	4 40Ca f mg/kg/day	5 Faeces Activity f@ %dose
1	55	M	48	Steat.	Osteop.	2 weeks	14.0	0.147	15.6	68.3
2	59	M	52	Diarrhoea	Normal	2 "	13.7+	0.140	15.8	52.6
3	50	M	72	Osteop.	Osteop.	2 "	30.0+	0.047	20.4	72.4
4	49	M	52	Steat.	Normal	3 "	19.3	0.100	22.6	64.5
5	65	F	67	Osteop.	Osteop.	3 "	30.0**	0.050	28.7	70.1
6	76	F	37	Osteop.	Osteop.	6 "	33.8+	0.081	30.1	86.5
	"	"	"	"	"	3 "	8.2	0.330	11.4	54.2
7	53	F	43	R.arthritis	Osteop.	7 "	11.7	0.200	8.9	49.9
	"	"	"	(steroids)	"	7 "	39.0+	0.062	32.7	76.6
8	43	F	62	Osteop.	Osteop.	3 "	26.5+	0.060	20.1	75.1
9	46	F	62	Osteop.	Osteop.	4 "	35.0+	0.047	29.5	75.2
10	64	M	51	Osteop.	Osteop.	3 "	5.0	0.390	6.8	66.1
	"	"	"	"	"	4 "	37.6+	0.052	33.7	72.0
11	60	F	57	Osteop.	Osteop.	2 "	4.3	0.420	5.4	64.4
	"	"	"	"	"	4 "	32.1	0.055	24.5	74.2
12	63	M	57	R.arthritis	Osteop.	3 "	4.5	0.400	5.4	30.0
	"	"	"	(steroids)	"	3 "	29.9++	0.059	21.0	55.3
	"	"	"	"	"	4 "	32.4+	0.054	23.3	58.5
13	53	F	66	Backache	Normal	3 "	1.7+	0.900	4.9	48.7
	"	"	"	"	"	3 "	16.9**	0.091	16.6	52.5
	"	"	"	"	"	3 "	16.9++	0.091	17.3	49.0
14	54	M	56	Osteop.	Osteop.	2 "	4.6	0.390	6.1	56.0
	"	"	"	"	"	3 "	21.7**	0.083	22.3	57.7
	"	"	"	"	"	4 "	20.5++	0.088	17.7	57.8

+ After 2 or 3 weeks equilibration
++ Calcium glycerophosphate supplements
** Calcium carbonate supplements
++ Calcium orthophosphate supplements
++ Calcium lactate supplements.

Continued overleaf /

TABLE XXXI CONTINUED:

Summary of all data broken down into separate studies according to the ^{40}Ca intake. Each value is the mean for the number of weeks shown in column 1.

	6	7	8	9	10	11	12	13	14
Case	S.A. f* %dose/mg	40Ca u mg/kg/day	Urine Activity u^0 %dose	S.A. u* %dose/mg	40Ca bal. b mg/kg/day	45Ca Ret. R %dose	B.F.R. m	B.R.R. d (1) mg/kg/day	B.R.R. d (2) mg/kg/day
1	0.092	1.4	3.9	0.058	-3.0	27.8	9.7	12.7	12.9
2	0.064	1.5	5.9	0.076	-3.6	41.5	10.5	14.1	14.0
3	0.050	3.4	8.9	0.037	+6.2	18.7	7.0	0.8	0.8
4	0.055	5.5	10.6	0.037	-8.8	24.9	13.0	21.8	21.8
5	0.371	1.1	5.4	0.073	+0.6	24.5	5.0	4.4	4.8
6	0.079	2.9	7.0	0.066	+0.8	6.5	2.7	1.9	2.9
	0.129	1.3	9.3	0.196	-4.5	36.5	5.1	9.6	9.6
7	0.130	2.2	12.1	0.130	+0.6	38.0	6.8	6.2	6.4
	0.050	2.2	7.6	0.080	+4.1	13.8	4.1	0.0	0.4
8	0.060	3.3	8.7	0.042	+3.1	16.2	9.0	5.9	3.3
9	0.042	3.0	7.7	0.042	+2.5	17.1	6.7	4.2	3.9
10	0.190	4.7	12.8	0.052	-6.5	21.1	8.0	14.5	11.5
	0.042	4.7	10.0	0.041	-0.8	18.0	8.7	9.5	6.8
11	0.210	1.2	2.3	0.034	-2.3	33.3	17.2	19.5	17.3
	0.053	0.8	1.6	0.035	+2.1	27.9	14.0	11.9	5.3
12	0.097	3.9	32.0	0.143	-4.8	38.0	4.6	9.4	9.5
	0.046	7.2	29.3	0.070	+1.7	15.4	3.9	2.2	2.3
	0.044	5.5	21.0	0.068	+3.4	20.5	5.3	1.9	2.7
13	0.135	2.0	16.2	0.123	-4.2	40.1	4.9	9.1	7.3
	0.048	3.0	13.9	0.070	-2.7	33.6	7.2	9.9	10.0
	0.043	2.4	12.5	0.080	-2.8	38.5	7.3	10.1	10.0
14	0.167	2.7	11.7	0.078	-4.2	32.3	7.4	11.6	11.5
	0.045	3.6	12.9	0.063	-4.2	29.4	8.4	12.6	12.6
	0.058	3.0	9.6	0.057	-0.2	32.6	10.2	10.4	10.5

S.A. = Specific activity
B.F.R. = Bone formation rate
B.R.R. = Bone resorption rate. (1) is calculated from the difference between balance and B.F.R. (2) Is calculated from the difference between the dietary and urinary specific activities.

TABLE XXXII

Mean percentage of administered dose of ^{45}Ca excreted during the first 8 weeks in faeces and urine, and mean retention of isotope.

Week	1	2	3	4	5	6	7	8
No. of obs.	6	13	18	17	12	12	12	12
Mean faecal excretion(%)	53.3	55.8	57.7	62.8	52.0	63.0	59.8	63.6
S.E.	7.4	3.5	4.7	4.3	5.5	7.2	6.4	6.5
Mean urinary excretion(%)	5.6	8.5	9.3	11.1	13.6	10.6	10.3	11.2
S.E.	1.2	2.1	1.7	1.9	3.3	1.9	1.9	1.8
Mean retention (%)	41.1	35.7	33.0	26.1	34.4	26.4	29.9	25.2

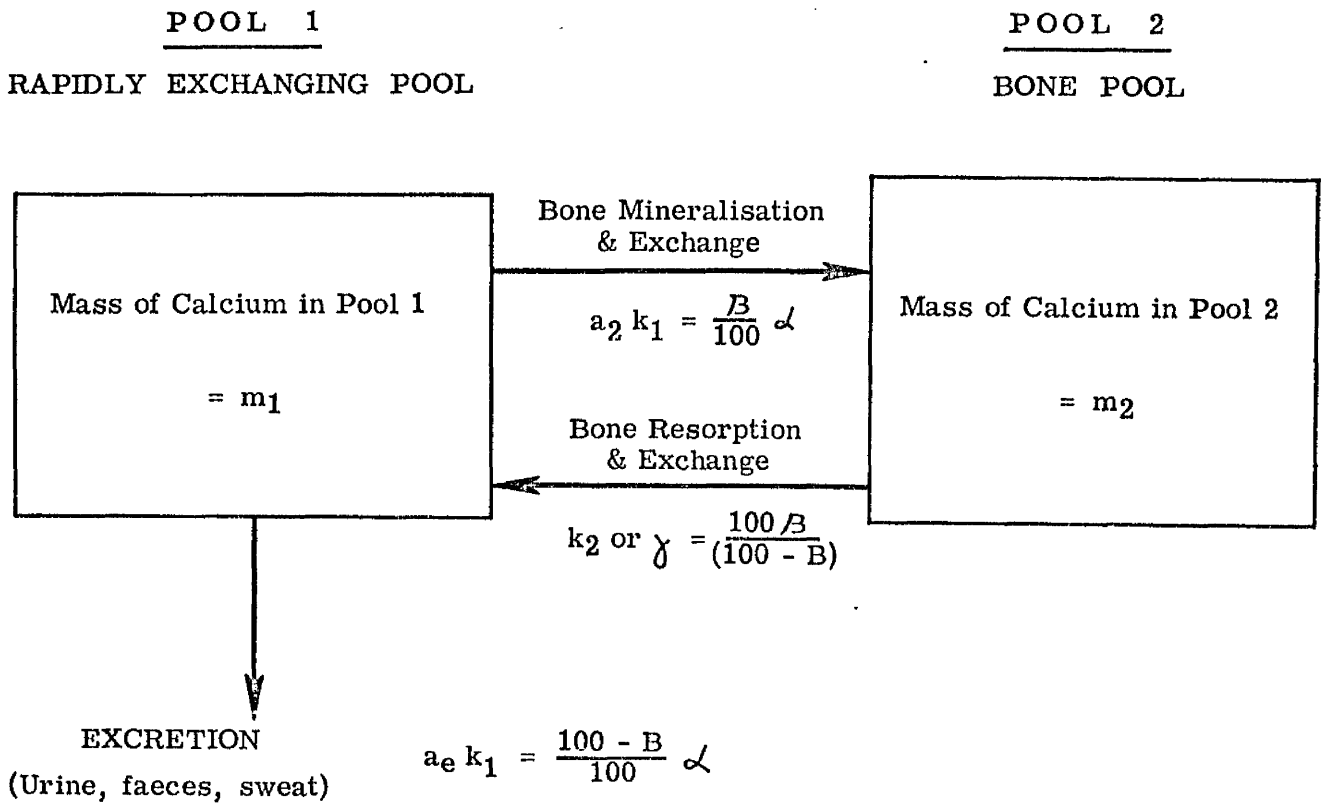


Fig 60 The two compartment model of showing the determination of the rate of return of isotope from bone (γ) by long-term exchange where after 40 days the retention $(R) = Be^{-\beta t}$ and the rate of return of isotope from bone $\gamma = \frac{100\beta}{100-B}$ (Shimmins, Allison, Smith and Speirs, 1967).

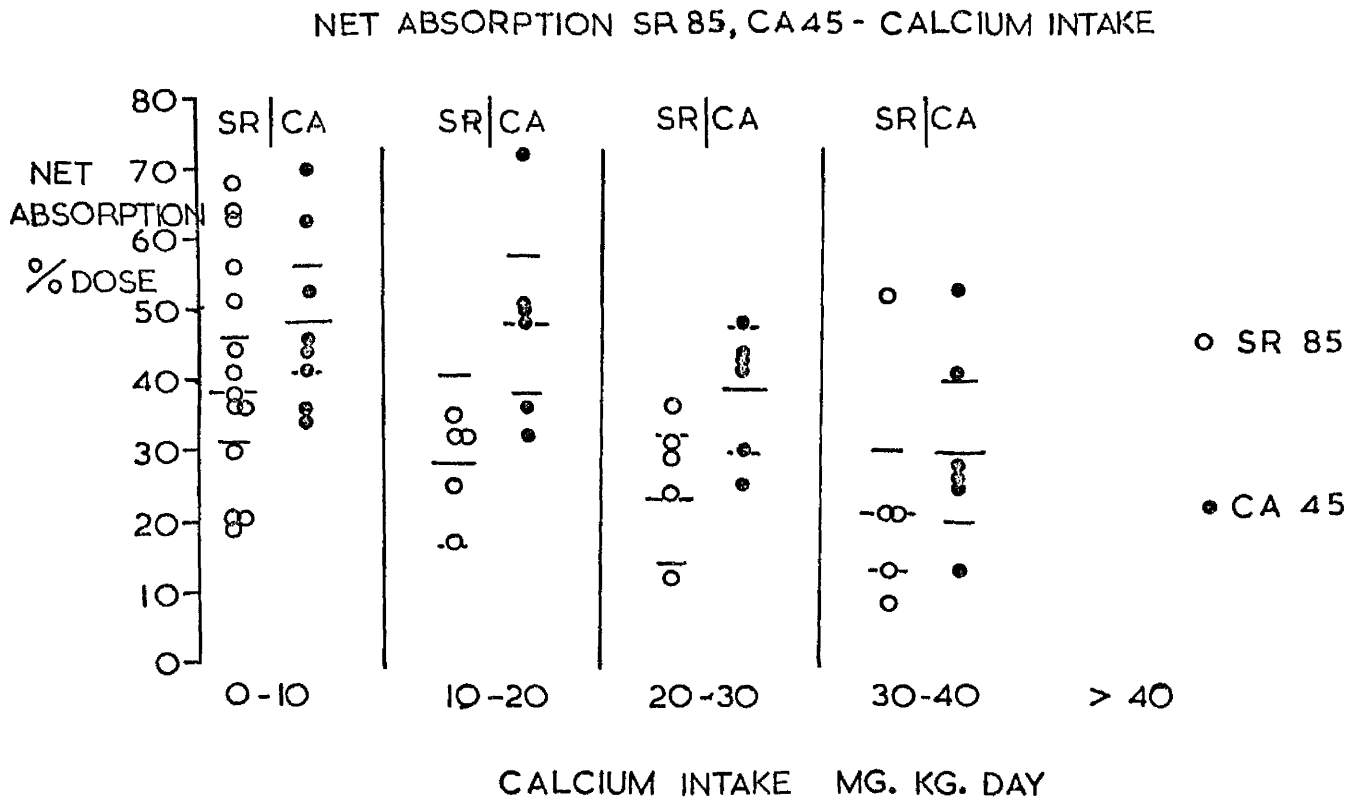


Fig 61 The net absorption of 45Ca and 85Sr at different levels of stable calcium intake during the continuous feeding of the isotopes. The mean and 2 S.E. ranges are shown.

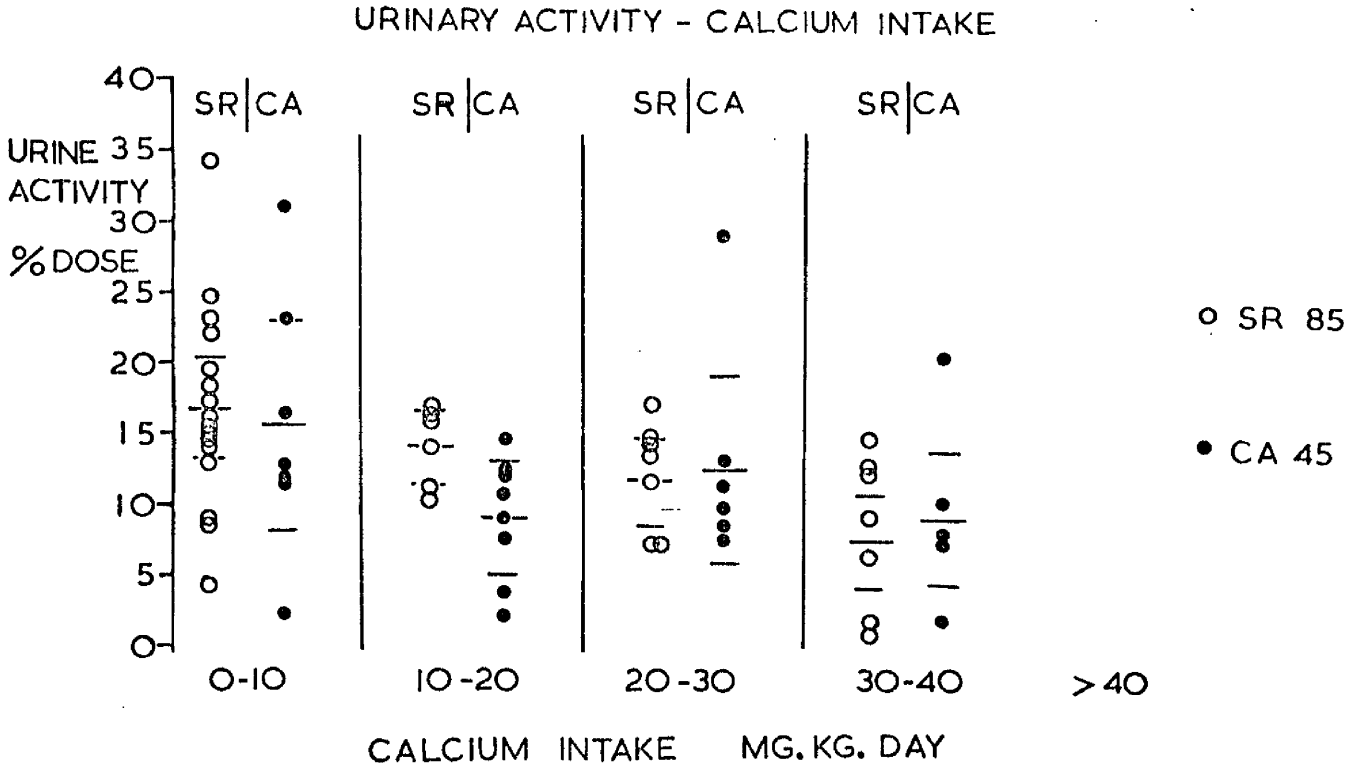


Fig 62 Urinary excretion of 45Ca and 85Sr at different levels of stable calcium intake during the continuous feeding of the isotopes. The mean and 2 S.E. ranges are shown.

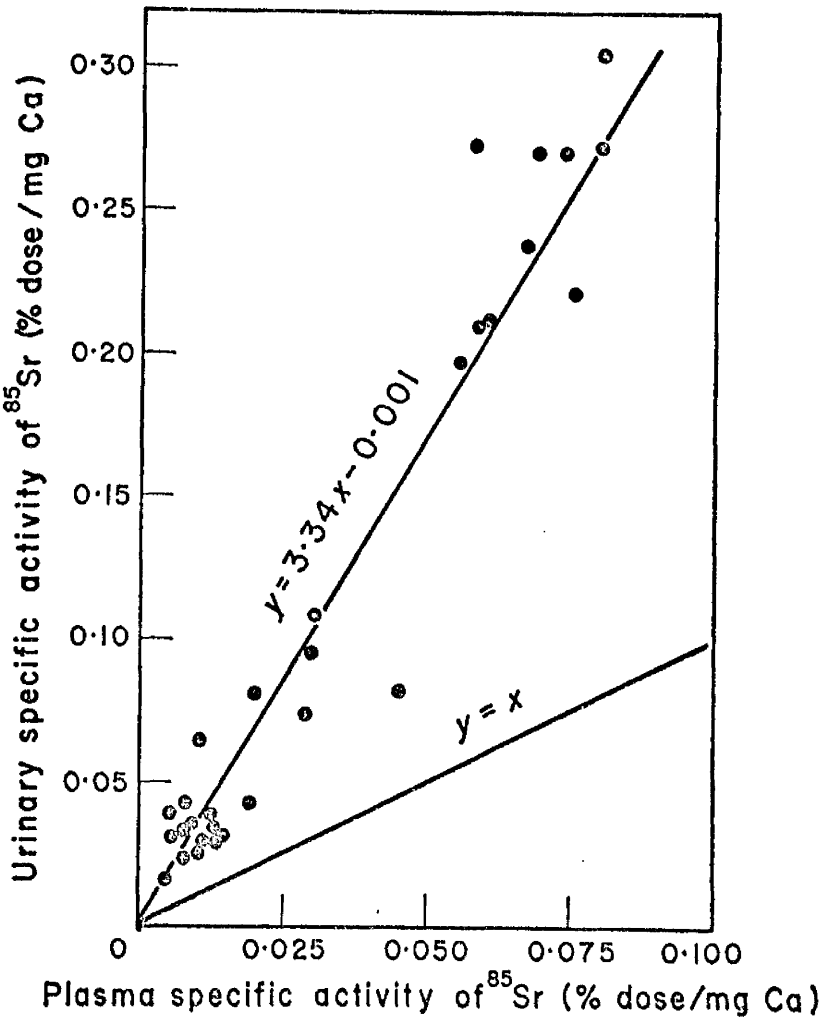


Fig 63 Discrimination against the reabsorption of 85Sr by the renal tubules obtained from the measurement of simultaneous plasma and urine samples.
Correlation coefficient r = 0.92.

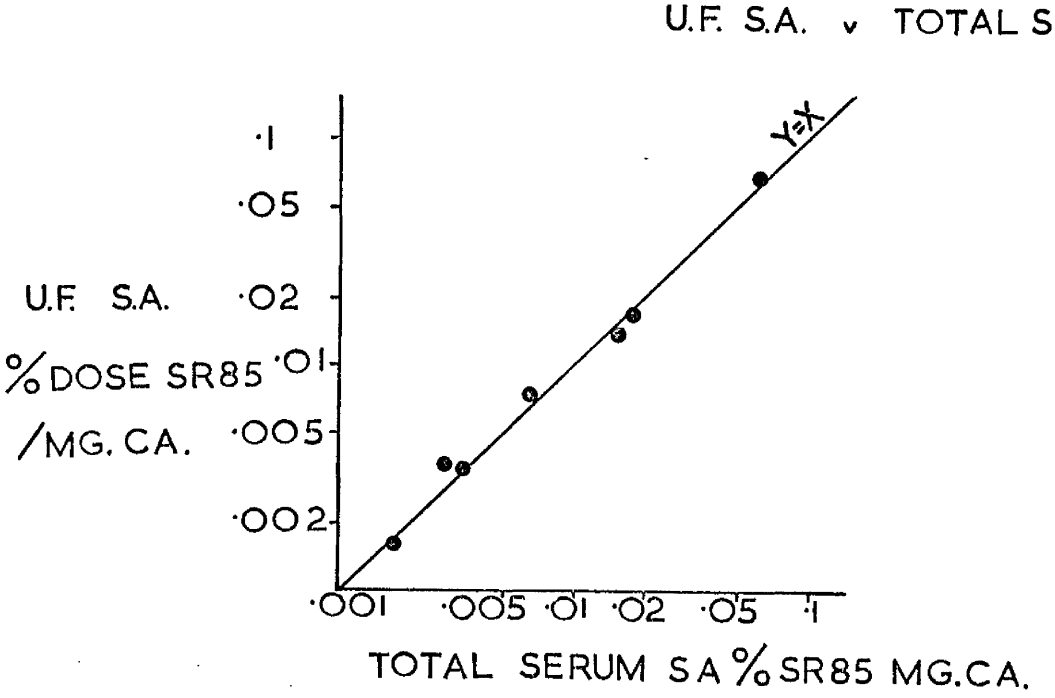


Fig 64 The serum and ultrafilterable 85Sr specific activity from the same serum samples. The specific activity is the same as that in the ultrafiltrate of the serum indicating no significant difference in the protein-binding of strontium compared with calcium.

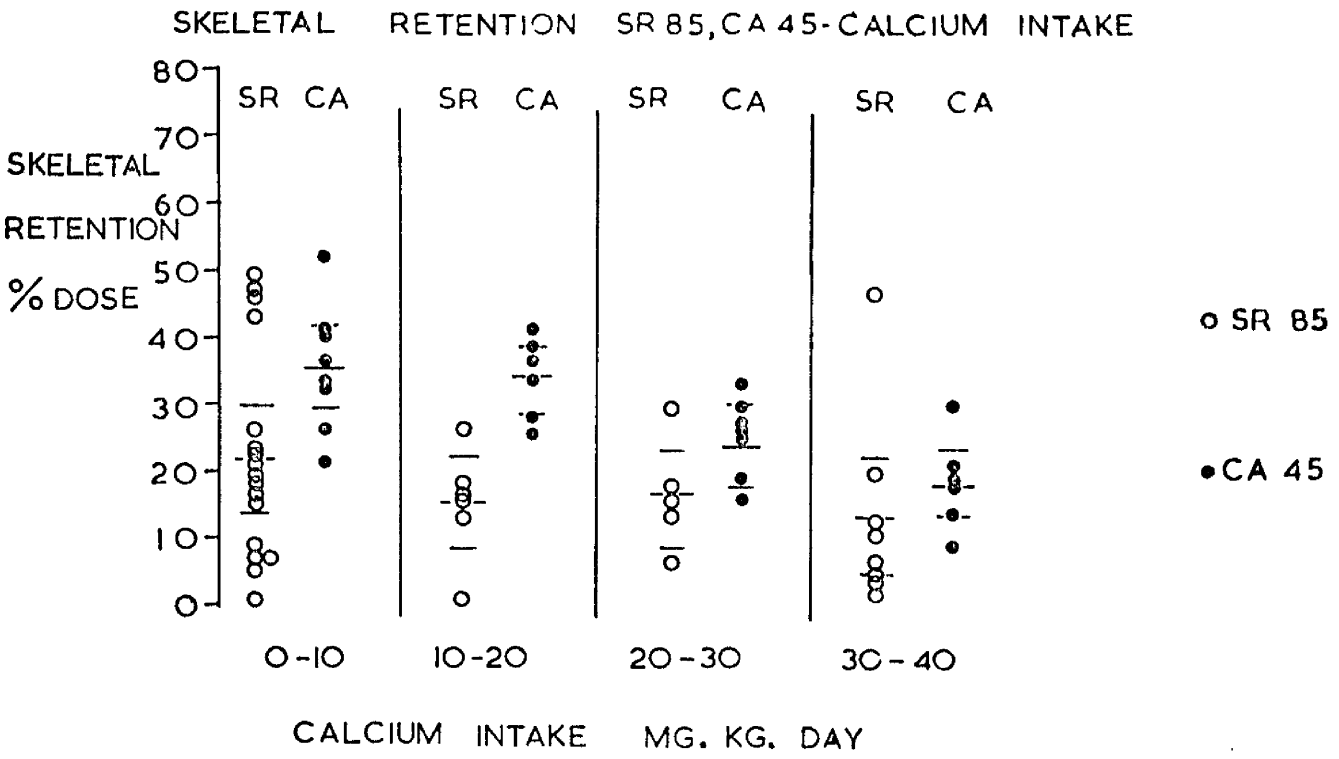


Fig 65 The skeletal retention of 45Ca and 85Sr at different levels of stable calcium intake during the continuous feeding of the isotopes. The mean and 2 S.E. ranges are shown.

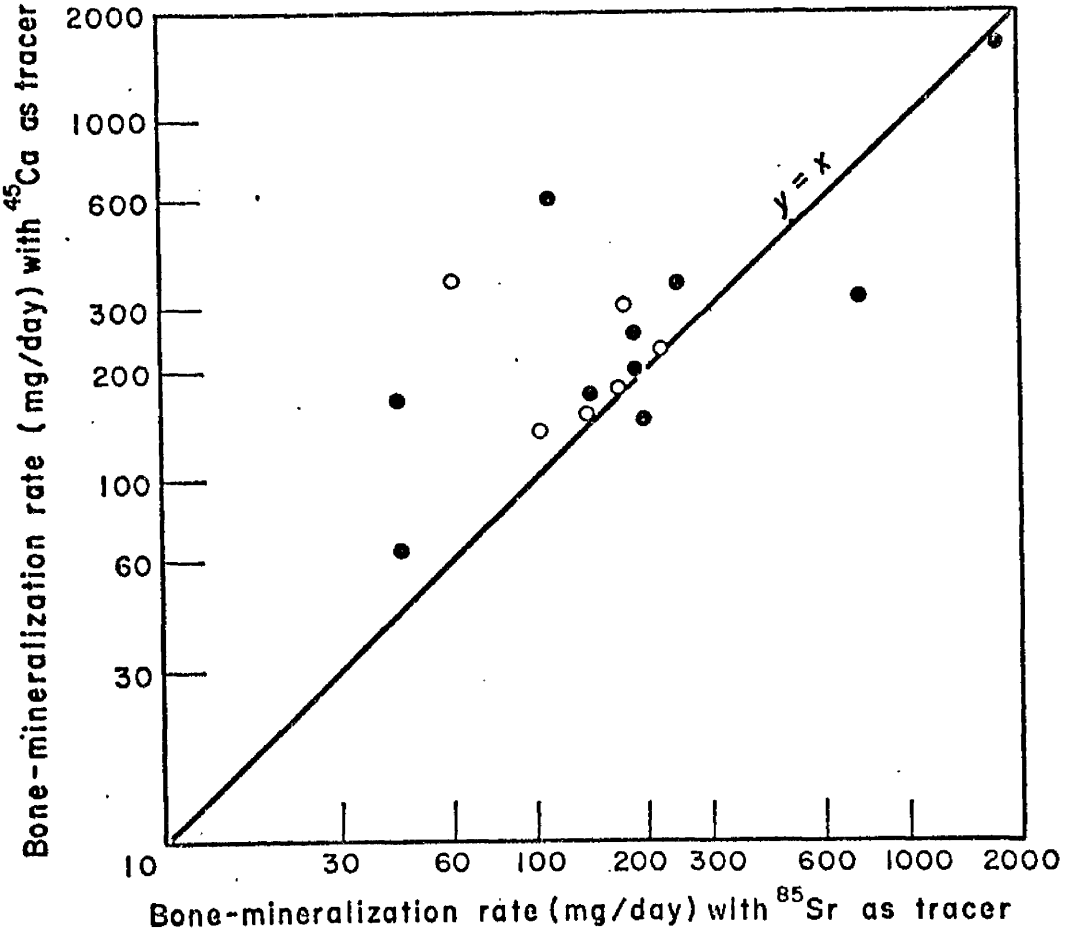


Fig 66 The bone mineral transfer rates in 6 patients in the present study (0) and 10 patients studied by Dow and Stanbury (1960) (⊙). The mineral transfer rate is estimated by the method of Bauer et al (1957) following the simultaneous intravenous administration of 45Ca and 85Sr.

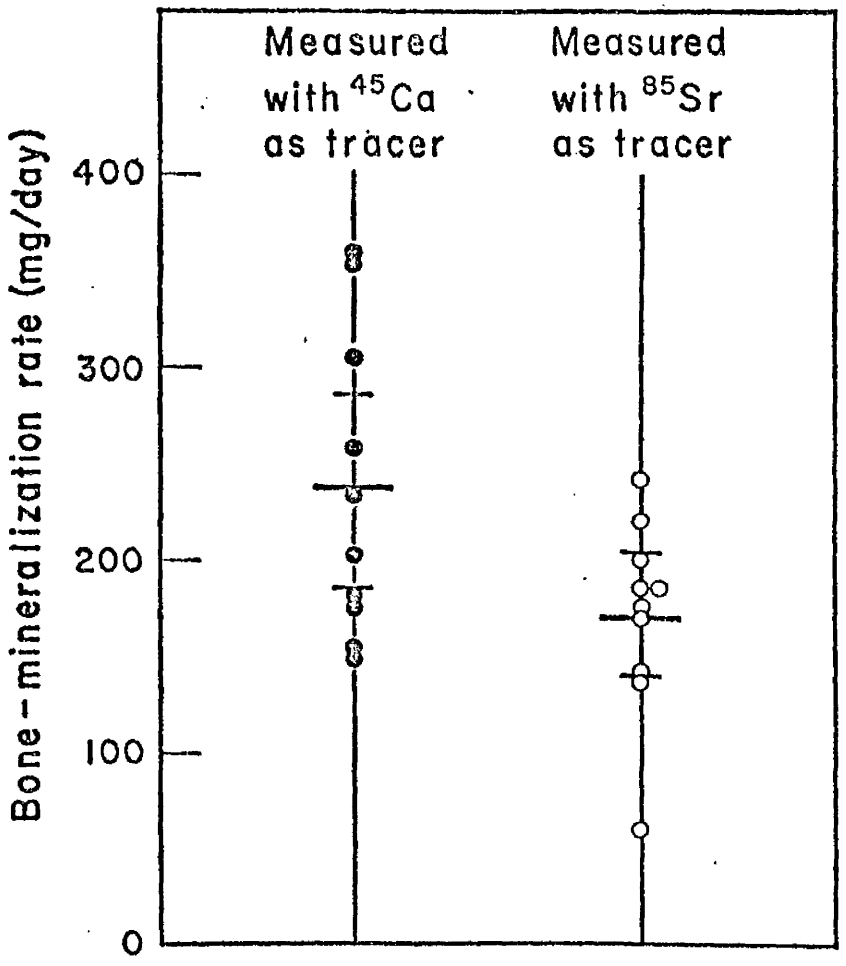


Fig 67 The bone mineralisation rates in the normal and osteoporotic subjects estimated from the simultaneous intravenous administration of 45Ca and 85Sr. The mean and 2 S.E. ranges are shown.

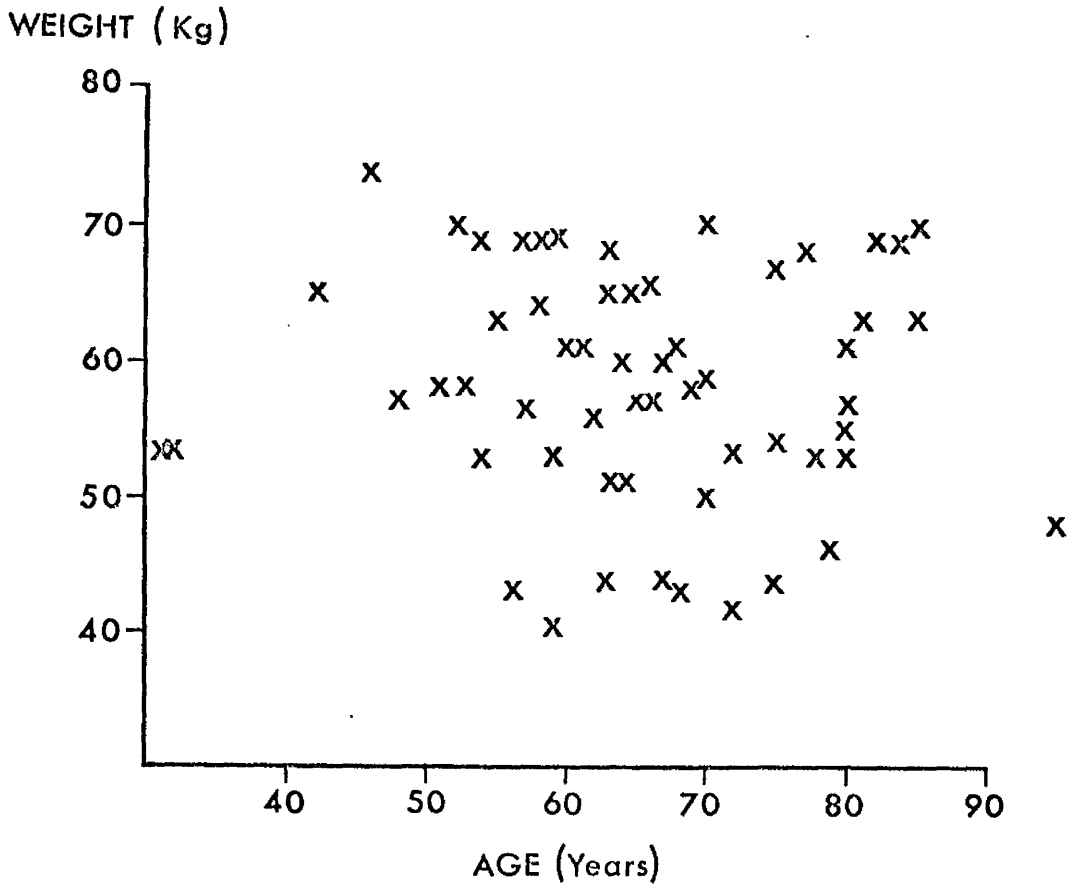


Fig 68 No significant fall in weight is apparent in the 65 female patients with age in whom the mineral transfer rate has been calculated.

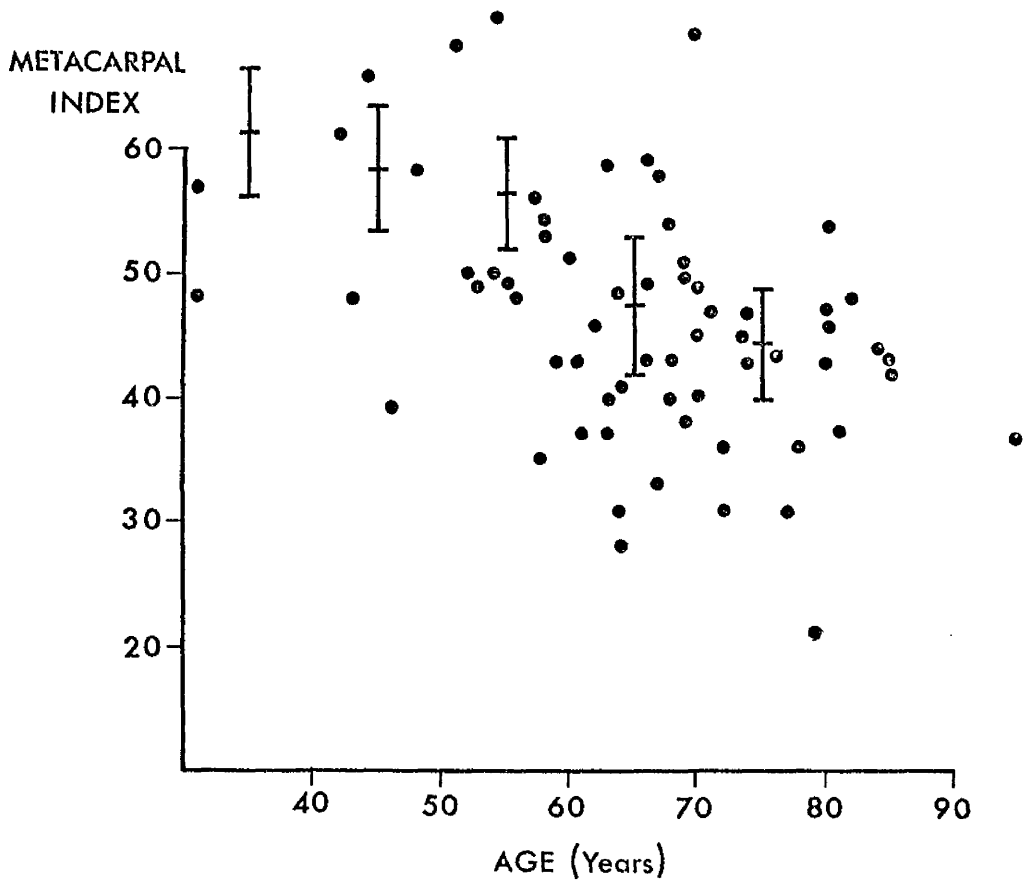


Fig 69 There is no significant difference in the Metacarpal Index (M.I.) between the patients studied and the normal subjects of Group II at any decade.

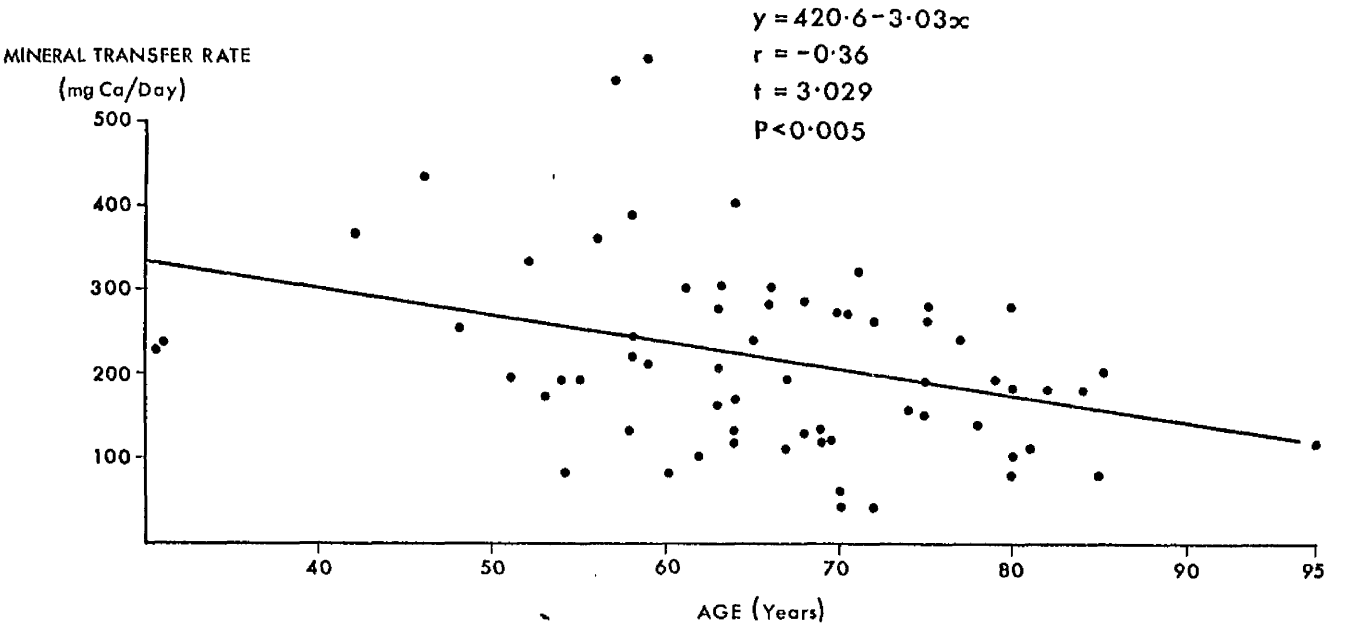


Fig 70 The mineral transfer rate plotted against age in 65 female subjects. The mineral transfer rate is calculated by the method of Bauer et al (1957) between 7 and 14 days after injection of 85Sr. There is a significant fall in the mineral transfer rate with age.

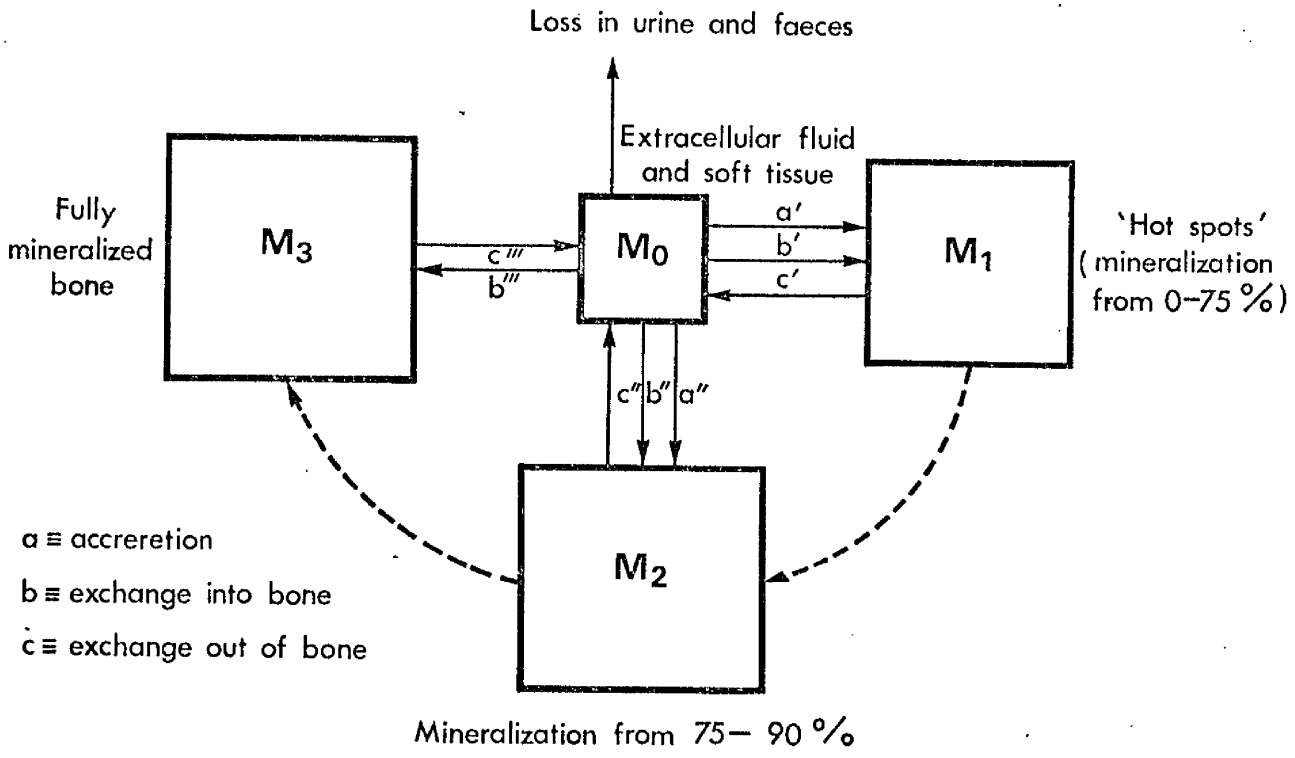


Fig 71 This figure illustrates the transfer of isotope from the E.C.F. into bone during the phase of conversion of freshly deposited matrix to fully calcified bone. Initial mineralisation up to about 75 percent occurs rapidly and proceeds much more slowly thereafter. In order to determine the true rate of mineralisation (i.e. the net gain of calcium) the magnitude of the exchange into bone would have to be known. There is no way of estimating this directly using simple isotope kinetic data.

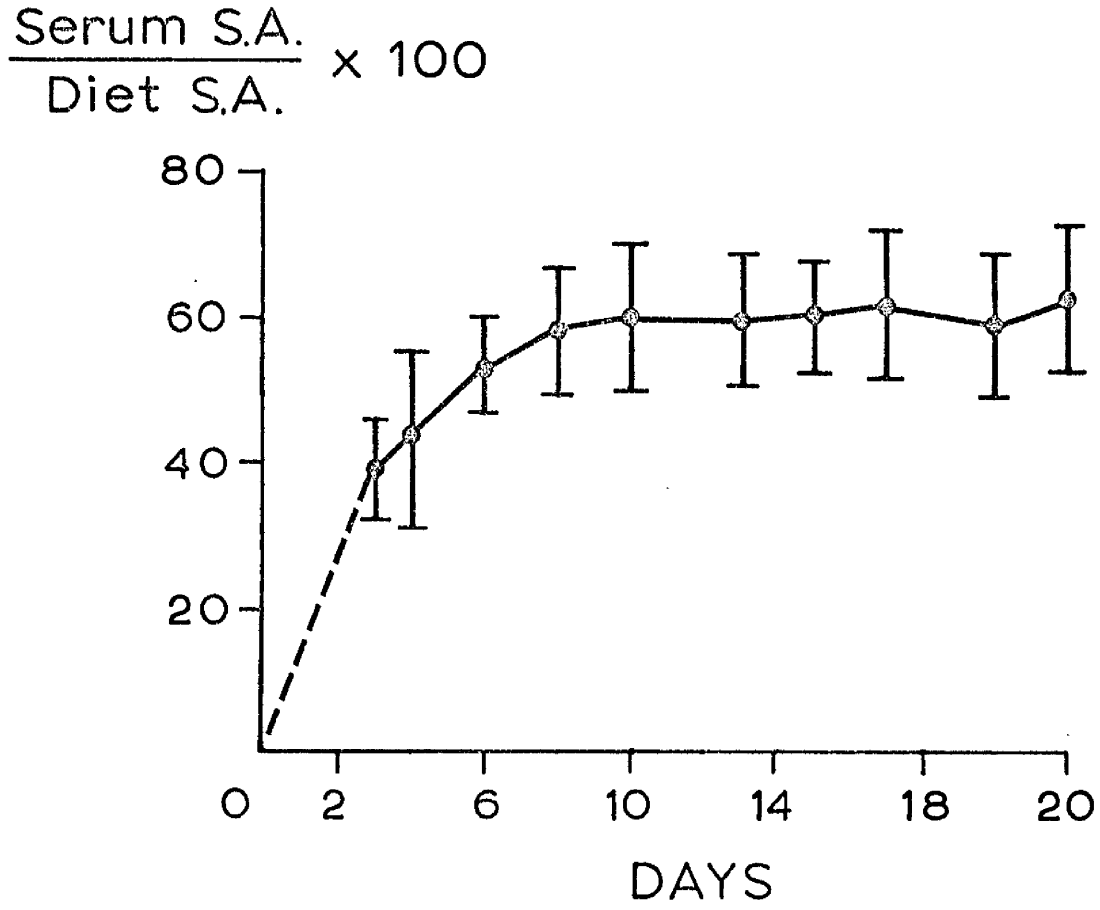


Fig 72 Mean plasma specific activities (as percentages of dietary specific activities) during the first 21 days of continuous feeding of 45Ca (± 1 S.E.).

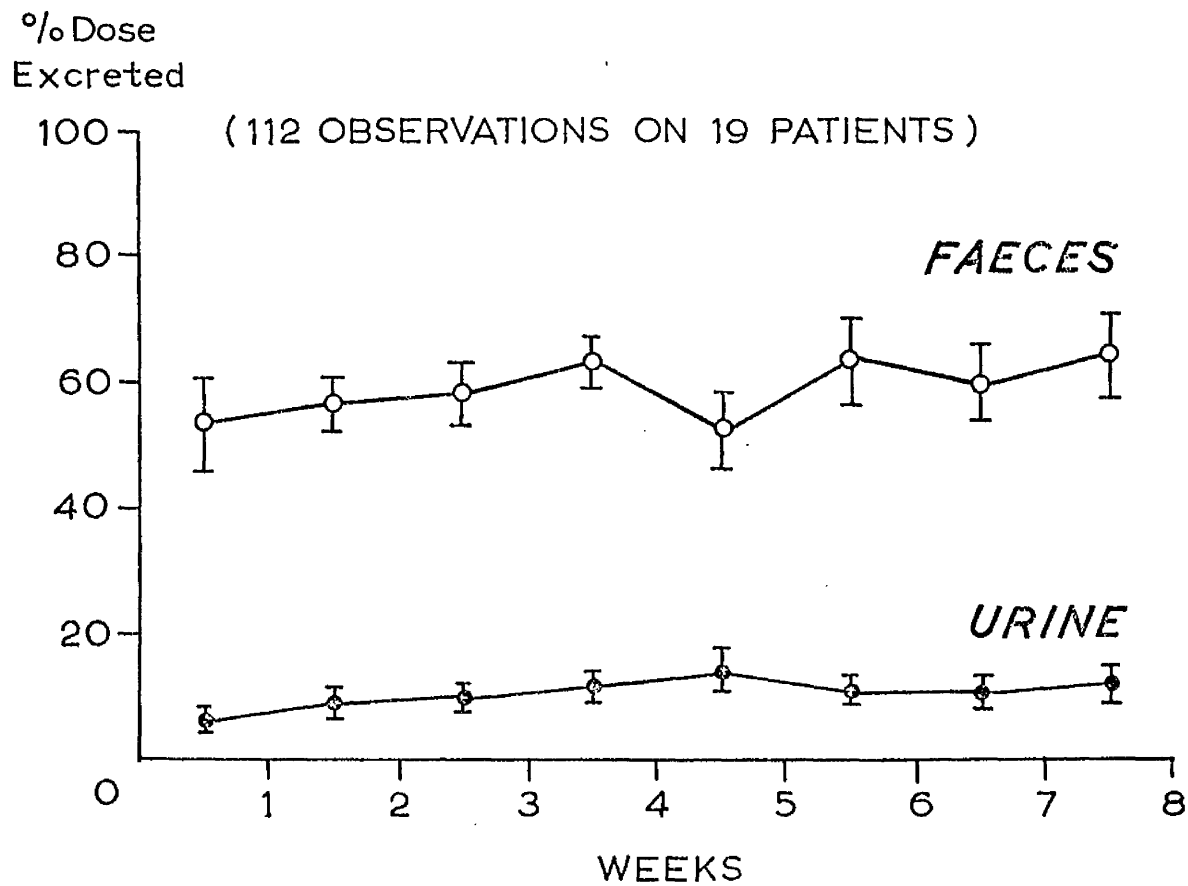


Fig 73 The percentage excretion of ^{45}Ca in faeces and urine during the first 8 weeks of continuous feeding.

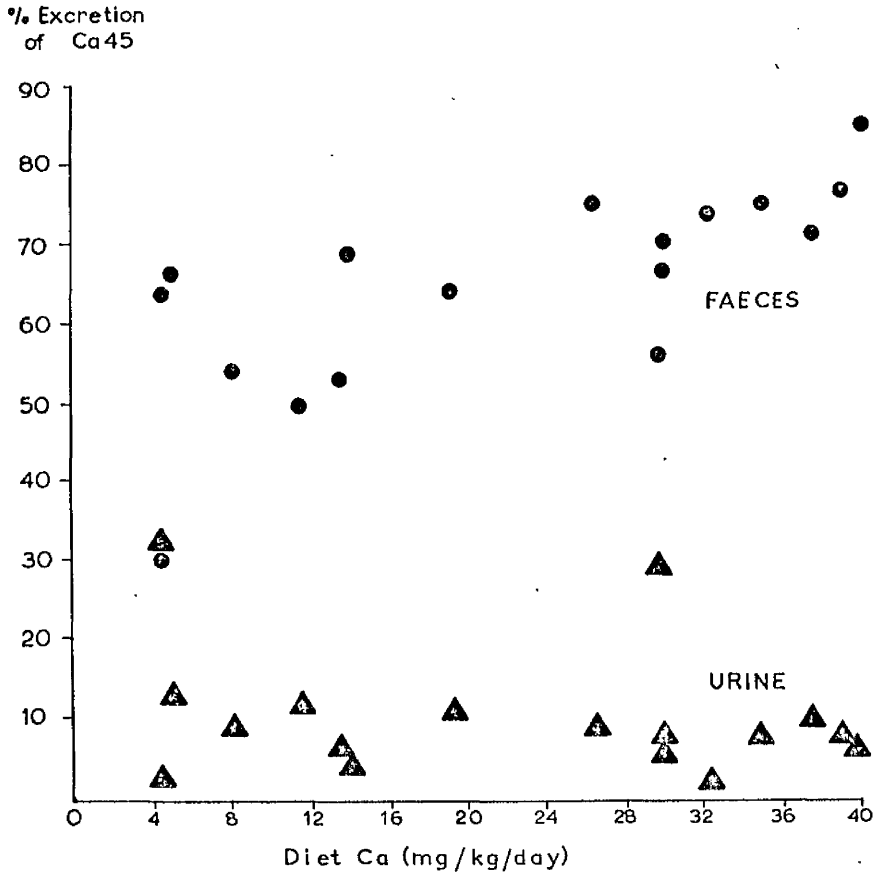


Fig 74 The excretion of ^{45}Ca in faeces and urine as a function of dietary calcium intake after two to three weeks equilibration.

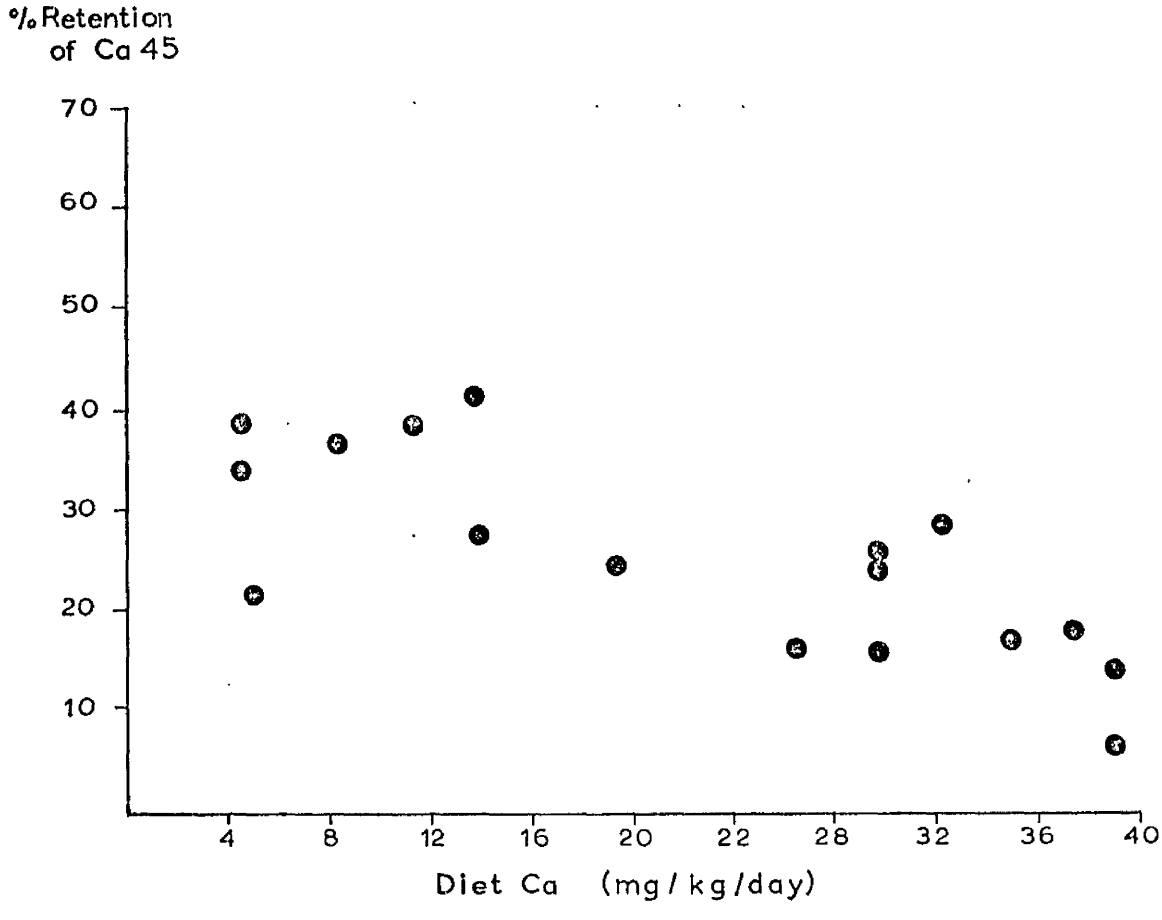


Fig 75 The percentage retention of 45Ca related to the dietary intake of calcium after two to three weeks equilibration.

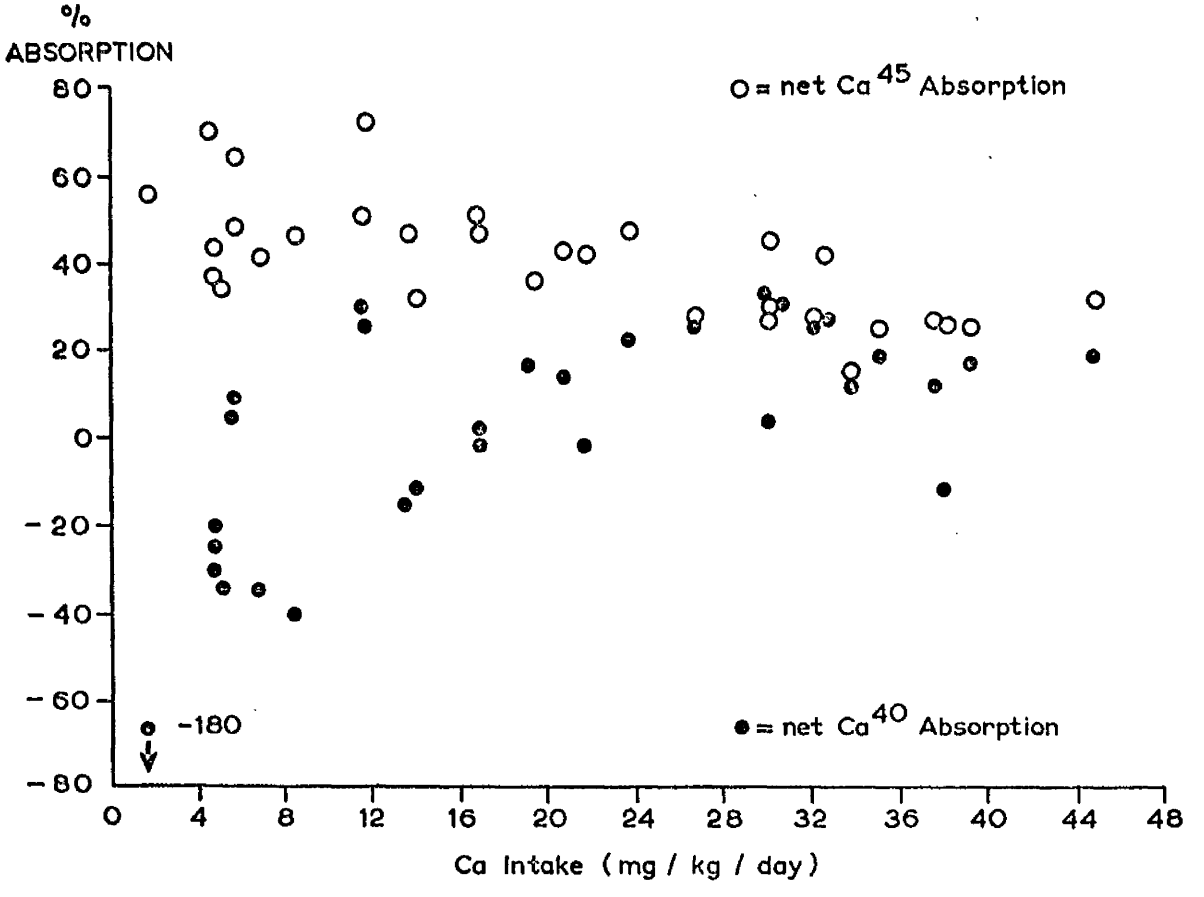


Fig 76 The percentage absorption of dietary calcium and 45Ca in relation to dietary calcium intake.

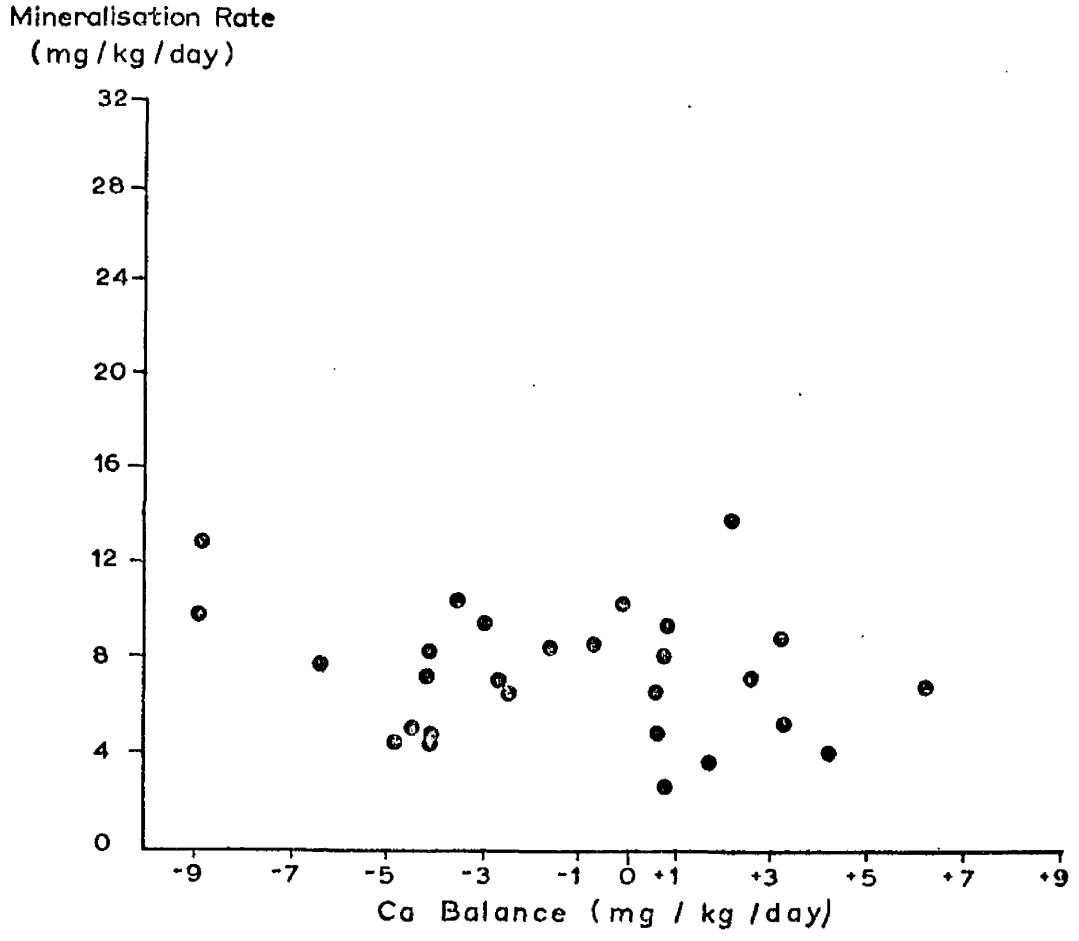


Fig 77 The mineral transfer rate into bone calculated from the continuous feeding of 45Ca.

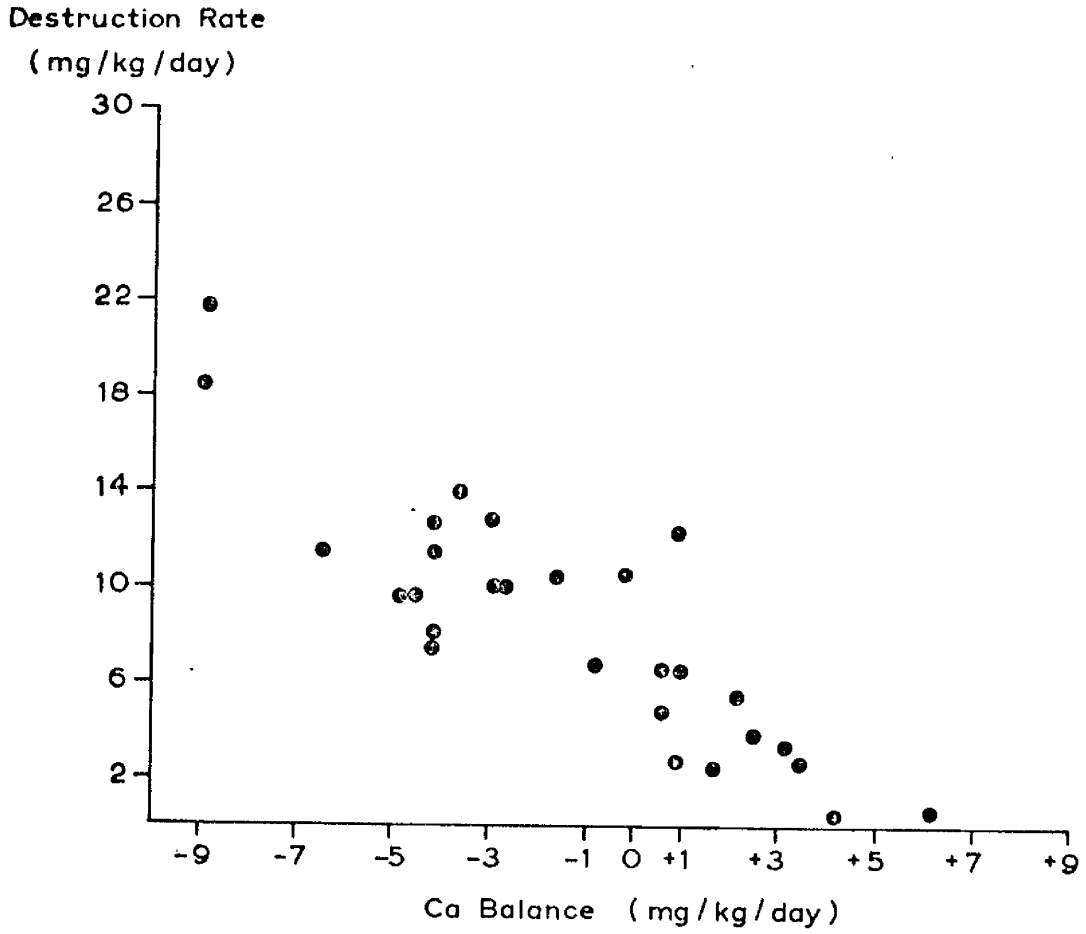


Fig 78 Bone destruction rate calculated from the continuous feeding of 45Ca in relation to calcium balance.

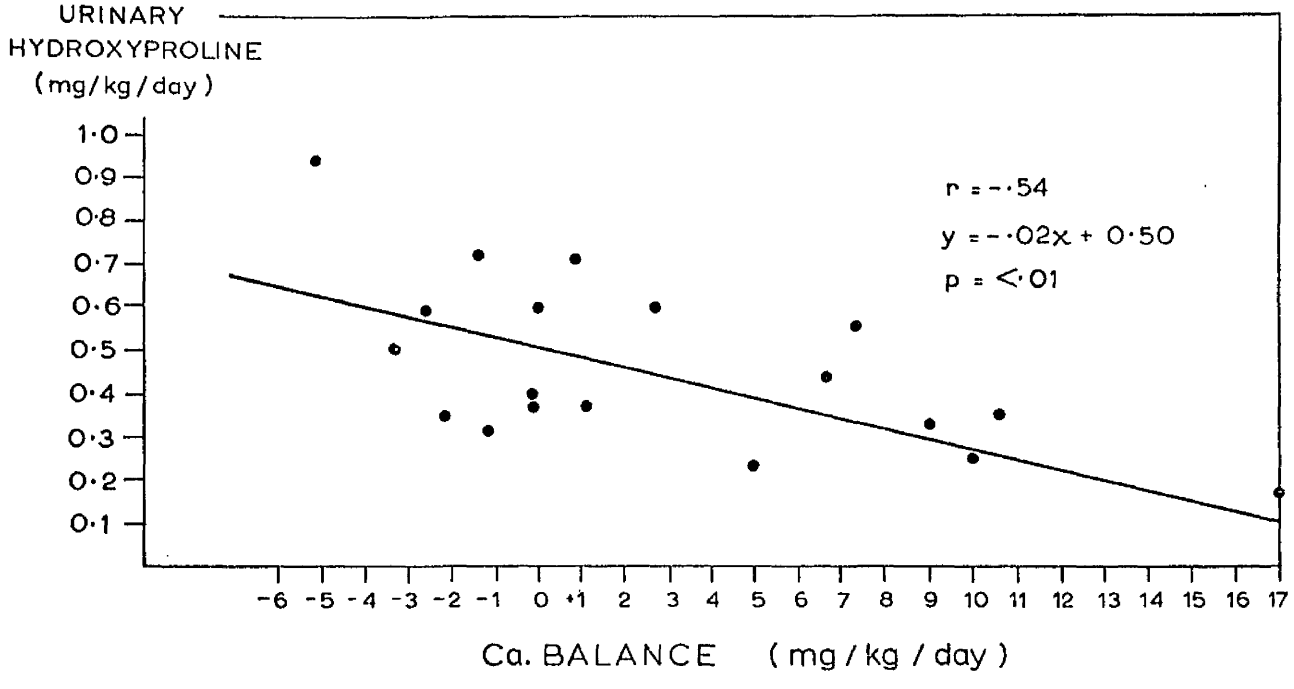


Fig 79 There is a significant inverse correlation between calcium balance and urinary hydroxyproline excretion.

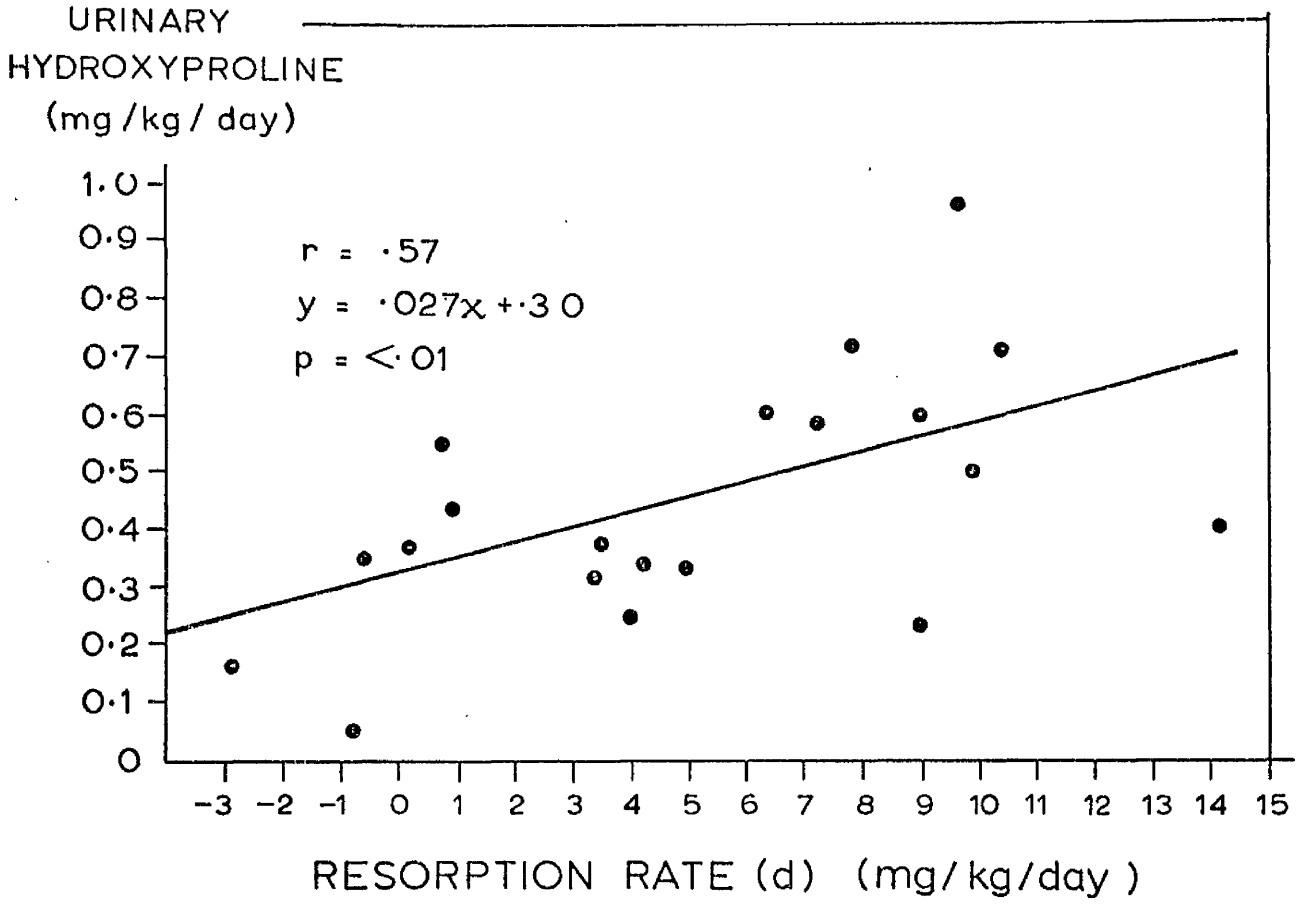


Fig 80 There is a significant direct correlation between bone resorption rate measured isotopically and urinary hydroxyproline output.

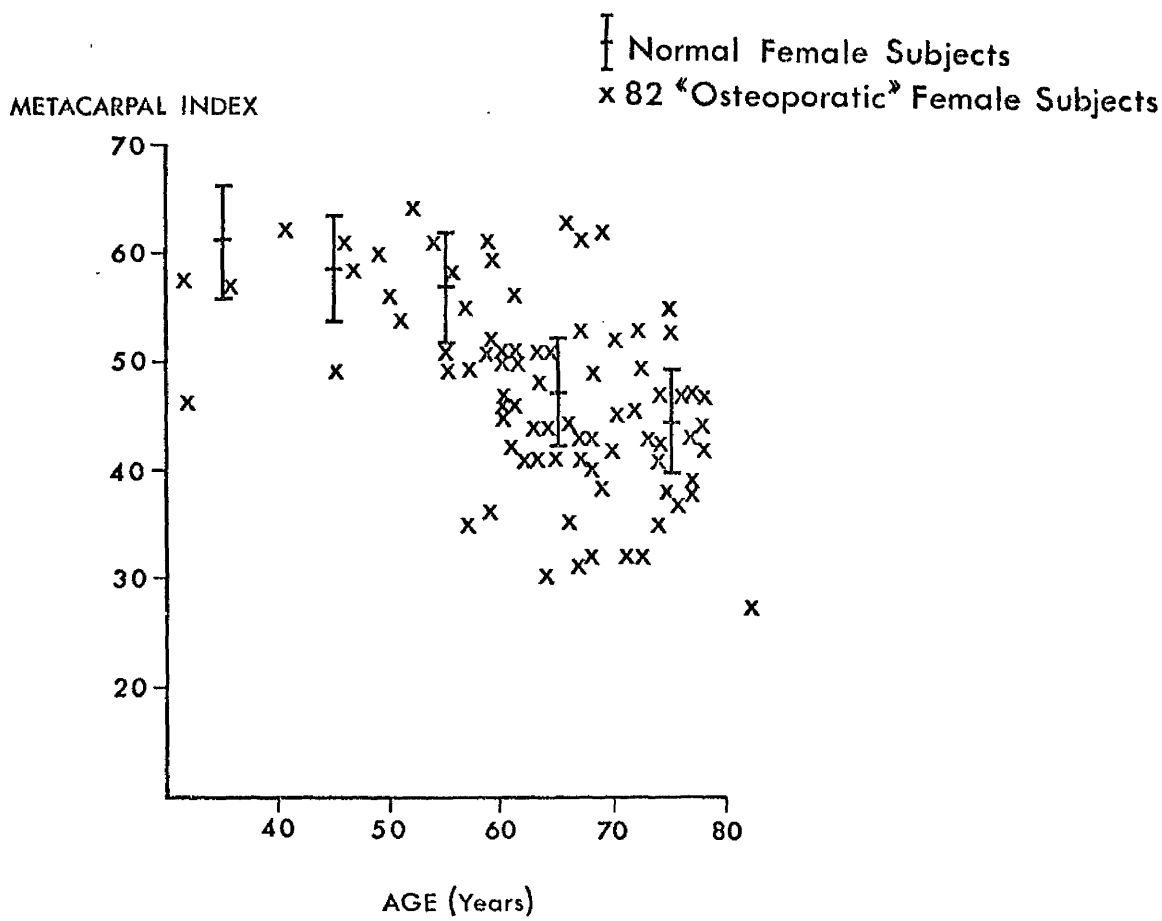


Fig 81 There is no significant difference between the Metacarpal Index of the normal and "osteoporotic" subjects at any decade.

Fig 82 Twenty-four hour urinary hydroxyproline excretion in 106 female subjects on a gelatin free diet plotted against age.

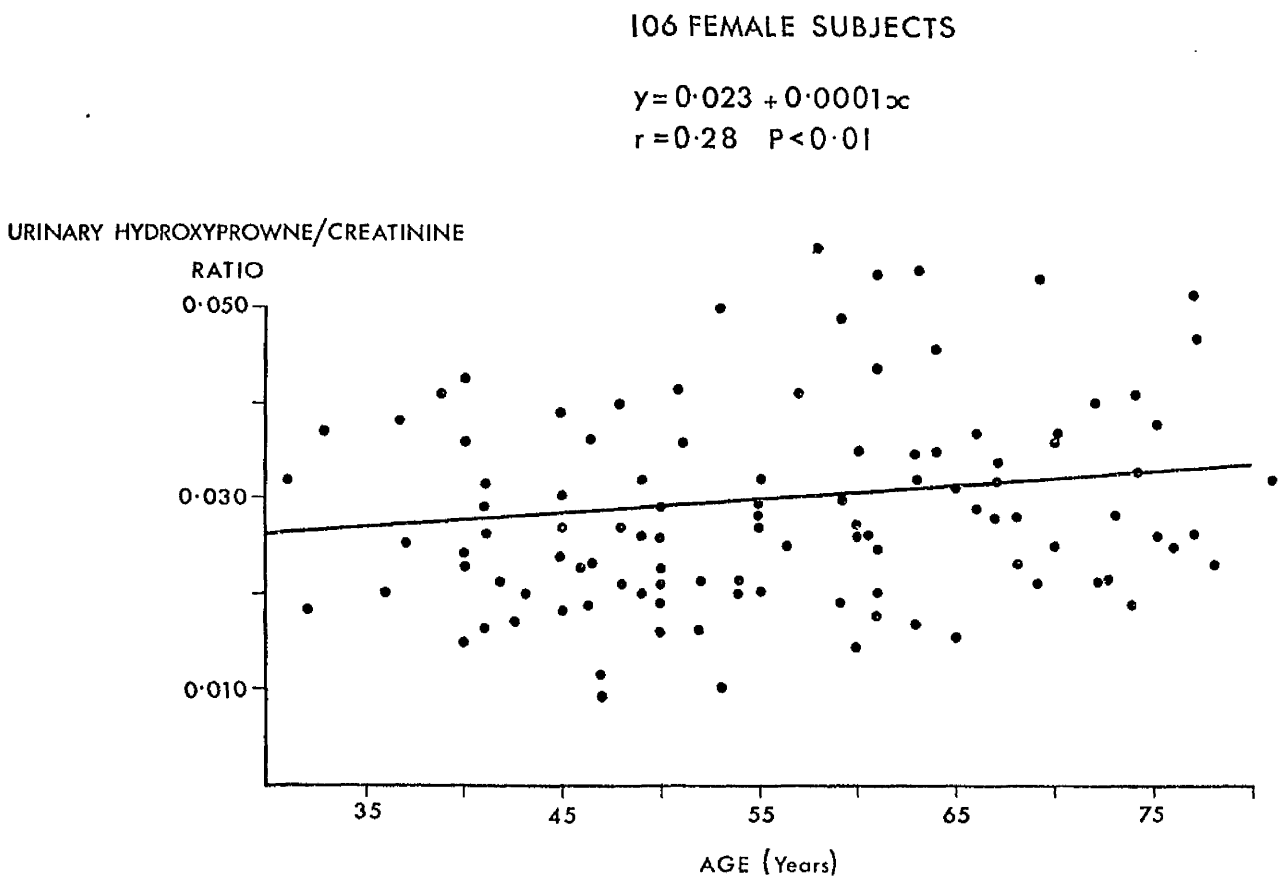


Fig 83 Twenty-four hour urinary hydroxyproline/creatinine ratio is directly related to age in women.

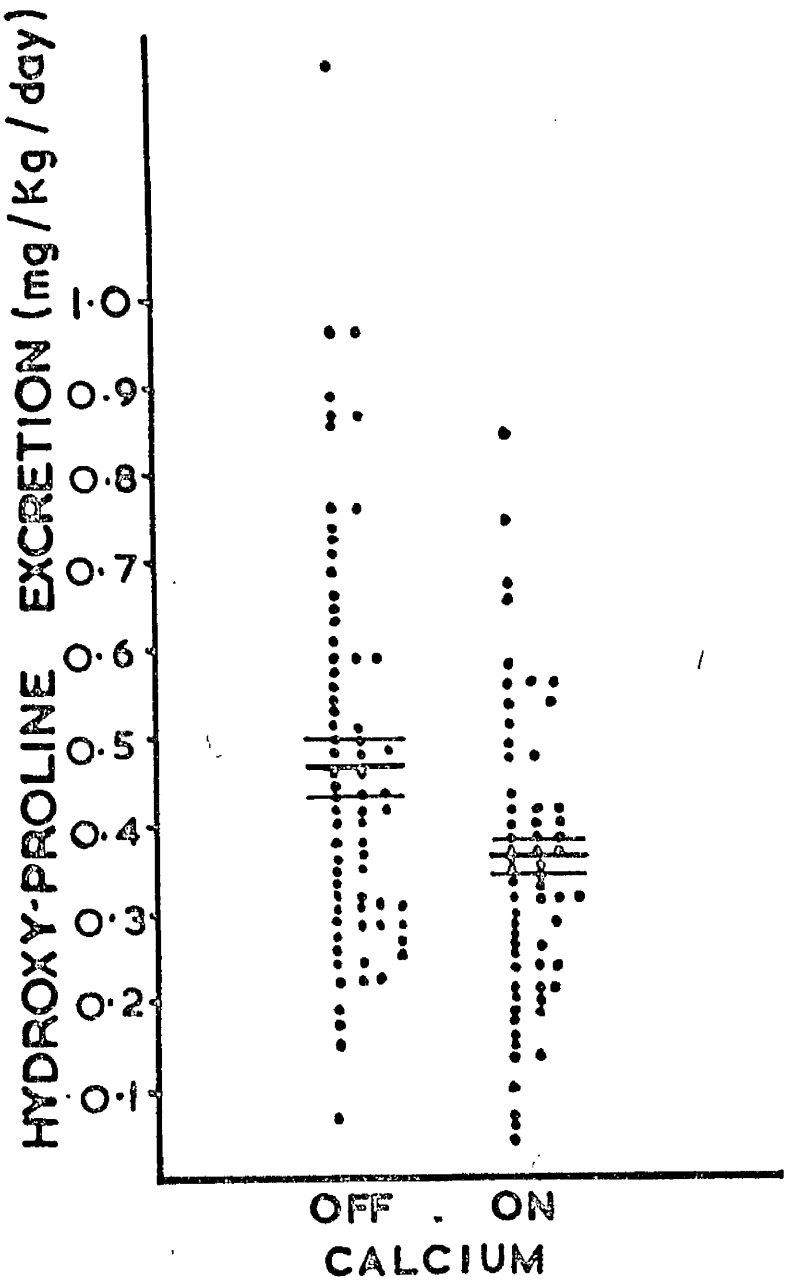


Fig 84 Urinary hydroxyproline excretion in 73 cases of osteoporosis untreated and in 64 on calcium glycerophosphate supplements. Fifty cases are common to both groups. The mean and 1 S.E. range are shown. The difference between the treated group is significant ($P < 0.01$).

CHAPTER VII

TABLE XXXIII

Social Classes

			Normal Females		Female 'Osteoporotic' Patients	
Class	1	Professional, etc.	6	4.3%	3	3.3%
"	2	Senior Executive	14	9.8%	9	10.0%
"	3	Skilled	96	68.1%	57	63.3%
"	4	Semi-skilled	18	12.8%	14	15.6%
"	5	Unskilled	7	5.0%	7	7.8%
Not recorded			11		7	

	WITH FRACTURES			WITHOUT FRACTURES		
	Upper limb	Lower limb	Other sites (ribs, etc.)	Upper limb	Lower limb	Other sites (ribs, etc.)
Normals	(9%)	(6.5%)	(1.4%)			
	12	9	2	134	137	144
Osteoporotics	(26%)	(6.6%)	(11.5%)			
	20	6	10	77	91	87

TABLE XXXIV

Incidence of fracture in normal subjects and 'osteoporotic' subjects.

TABLE XXXV

Site and incidence of backache in normal subjects and in 'osteoporotic' patients.

	Normals		'Osteoporotics'	
Site of backache	Number	Percentage	Number	Percentage
Cervical	0	0	2	2.0%
Thoracic	2	1.3%	7	7.3%
Thoraco-lumbar	1	0.7%	6	6.2%
Lumbar	33	21.5%	44	45.0%
Lumbar and legs	10	6.6%	18	18.7%
Sacral	6	3.9%	12	12.4%
Girdle thoracic	0	0	0	0
Girdle lumbar	0	0	0	0

	Group I (female)	Group II (male)	Group III (female)	Group IV (male)
31 - 40	17	12	18	8
41 - 50	44	21	25	19
51 - 60	34	14	19	13
61 - 70	28	11	12	9
71 - 80	28	8	10	10

TABLE XXXVI

The percentage of normal male and female subjects at each decade with backache.

TABLE XXXVII

The incidence of symptoms other than backache or bone pain in the normal subjects and 'osteoporotic' subjects.

	NORMALS		'OSTEOPOROTICS'	
	Number	%age	Number	%age
Diarrhoea	4	2.6	4	4.1
Gastrectomy	0	0	0	0
Short-circuiting operation	1	0.7	0	0
Renal colic	1	0.7	3	3.1
Single renal stone	2	1.4	2	2.0
Recurrent renal stones	1	0.7	0	0
Frequency of micturition	14	9.2	31	32.0
Nocturia	39	25.6	43	44.0
Dysuria	13	8.6	23	24.0
Hysterectomy	8	5.3	7	7.2
Oophorectomy	2	1.3	13	13.4

TABLE XXXVIII

Parity by decades

Age	Normal Females	'Osteoporotic' Females
21 - 30	1.7	2.0
31 - 40	2.1	1.5
41 - 50	2.3	2.4
51 - 60	2.0	2.2
61 - 70	3.1	2.4
71 - 99	1.9	2.3

SUBJECTS	Number of subjects	X-ray indices	Corr. coef. (r)	P
Normals	145	Femoral	-0.40	P < 0.001
	142	Spine	-0.26	P < 0.005
	145	Hand	-0.64	P < 0.001
	136	R.V.D.	-0.50	P < 0.001
'Osteoporotics'	97	Femoral	-0.36	P < 0.001
	97	Spine	Not significant	
	97	Hand	-0.59	P < 0.001
	97	R.V.D.	-0.40	P < 0.001
Normals and 'Osteoporotics'	242	Femoral	-0.46	P < 0.001
	239	Spine	-0.30	P < 0.001
	242	Hand	-0.67	P < 0.001
	233	R.V.D.	-0.55	P < 0.001

TABLE XXXIX

Correlation analysis of x-ray indices with age

SUBJECTS	Number of subjects	Daily intake: Dietary Constituent	Daily intake: Corr. coef. (r)	Daily intake: P	Daily intake/Kg body weight: Corr. coef. (r)	Daily intake/Kg body weight: P
Normals	145	Calcium	Not significant		Not significant	
	145	Phosphorus	-0.2	P < 0.02	Not significant	
	145	Protein	-0.2	P < 0.005	Not significant	
	145	Vitamin D	-0.18	P < 0.05	P < 0.05	
'Osteoporotics'	96	Calcium	Not significant		Not significant	
	96	Phosphorus	Not significant		Not significant	
	96	Protein	-0.25	P < 0.02	Not significant	
	96	Vitamin D	Not significant		Not significant	
Normals and 'Osteoporotics'	241	Calcium	-0.14	P < 0.05	Not significant	
	241	Phosphorus	-0.26	P < 0.001	- 0.14	P < 0.05
	241	Protein	-0.36	P < 0.001	-0.22	P < 0.001
	241	Vitamin D	-0.22	P < 0.001	-0.21	P < 0.005

TABLE XL

Correlation analysis of dietary constituents and dietary constituents/Kg body weight with age

	Number of subjects	r	T-value	P		Number of subjects	r	T-value	P
Calcium					Calcium/body weight				
F.I.	145	0.061	0.731	n.s.	F.I.	145	-0.042	-0.508	n.s.
S.I.	145	-0.007	-0.085	n.s.	S.I.	145	-0.103	-1.240	n.s.
M.I.	145	0.168	2.035	< 0.05	M.I.	145	0.126	1.523	n.s.
R.V.D.	142	0.130	1.549	n.s.	R.V.D.	142	0.055	0.654	n.s.
Phosphorus					Phosphorus/body weight				
F.I.	145	0.137	1.648	n.s.	F.I.	145	0.002	0.029	n.s.
S.I.	145	0.021	0.246	n.s.	S.I.	145	-0.097	-1.169	n.s.
M.I.	145	0.234	2.881	< 0.005	M.I.	145	0.175	2.129	< 0.05
R.V.D.	142	0.214	2.596	< 0.02	R.V.D.	142	0.087	1.029	n.s.
Protein					Protein/body weight				
F.I.	145	0.202	2.462	< 0.02	F.I.	145	0.046	0.551	n.s.
S.I.	145	0.083	0.994	n.s.	S.I.	145	-0.056	-0.667	n.s.
M.I.	145	0.275	3.422	< 0.005	M.I.	145	0.209	2.555	< 0.02
R.V.D.	142	0.196	2.360	< 0.025	R.V.D.	142	0.087	1.029	n.s.
Vitamin D					Vitamin D/body weight				
F.I.	145	0.082	0.982	n.s.	F.I.	145	0.030	0.355	n.s.
S.I.	145	0.180	2.185	< 0.05	S.I.	145	0.105	1.258	n.s.
M.I.	145	0.118	1.421	n.s.	M.I.	145	0.127	1.531	n.s.
R.V.D.	142	0.199	2.403	< 0.02	R.V.D.	142	0.185	2.228	< 0.05

TABLE XLI

The relation between X-ray indices and diet, and diet divided by body weight in normal female subjects. (n.s. = not significant)

	Number of subjects	r	T-value	P		Number of subjects	r	T-value	P
Calcium					Calcium/body weight				
F.I.	97	0.050	0.491	n.s.	F.I.	93	-0.052	-0.495	n.s.
S.I.	94	-0.038	-0.365	n.s.	S.I.	91	-0.102	-0.972	n.s.
M.I.	97	0.231	2.317	< 0.025	M.I.	93	0.116	1.113	n.s.
R.V.D.	91	0.105	0.995	n.s.	R.V.D.	87	0.045	0.413	n.s.
Phosphorus					Phosphorus/body weight				
F.I.	97	0.052	0.503	n.s.	F.I.	93	-0.105	-1.006	n.s.
S.I.	94	0.009	0.090	n.s.	S.I.	91	-0.064	-0.608	n.s.
M.I.	97	0.216	2.159	< 0.05	M.I.	93	0.065	0.625	n.s.
R.V.D.	91	0.125	1.190	n.s.	R.V.D.	87	0.043	0.393	n.s.
Protein					Protein/body weight				
F.I.	97	0.132	1.302	n.s.	F.I.	93	-0.077	-0.739	n.s.
S.I.	94	0.128	1.237	n.s.	S.I.	91	0.029	0.274	n.s.
M.I.	97	0.165	1.632	n.s.	M.I.	93	-0.004	-0.035	n.s.
R.V.D.	91	0.168	1.606	n.s.	R.V.D.	87	0.057	0.529	n.s.
Vitamin D					Vitamin D/body weight				
F.I.	97	0.010	0.101	n.s.	F.I.	93	-0.079	-0.753	n.s.
S.I.	94	0.095	0.914	n.s.	S.I.	91	0.090	0.849	n.s.
M.I.	97	0.034	0.331	n.s.	M.I.	93	0.014	0.137	n.s.
R.V.D.	91	0.090	0.854	n.s.	R.V.D.	87	0.073	0.674	n.s.

TABLE XLII

The relation between x-ray indices and diet and diet divided by body weight in 'osteoporotic' female subjects (n.s. - not significant).

	Number of subjects	r	T-value	P		Number of subjects	r	T-value	P
Calcium					Calcium/body weight				
F.I.	242	0.058	0.904	n.s.	F.I.	238	-0.062	-0.962	n.s.
S.I.	239	-0.022	-0.336	n.s.	S.I.	236	-0.120	-1.856	n.s.
M.I.	242	0.191	3.015	< 0.005	M.I.	238	0.091	1.404	n.s.
R.V.D.	233	0.105	1.600	n.s.	R.V.D.	229	0.017	0.255	n.s.
Phosphorus					Phosphorus/body weight				
F.I.	242	0.150	2.357	< 0.02	F.I.	238	-0.024	-0.375	n.s.
S.I.	239	0.090	1.394	n.s.	S.I.	236	-0.046	-0.698	n.s.
M.I.	242	0.277	4.462	< 0.001	M.I.	238	0.142	2.200	< 0.05
R.V.D.	233	0.224	3.498	< 0.001	R.V.D.	229	0.091	1.369	n.s.
Protein					Protein/body weight				
F.I.	242	0.265	4.258	< 0.001	F.I.	238	0.055	0.850	n.s.
S.I.	239	0.238	3.772	< 0.001	S.I.	236	0.071	1.096	n.s.
M.I.	242	0.328	5.378	< 0.001	M.I.	238	0.182	2.836	< 0.01
R.V.D.	233	0.283	4.483	< 0.001	R.V.D.	229	0.131	1.991	< 0.05
Vitamin D					Vitamin D/body weight				
F.I.	242	0.121	1.888	n.s.	F.I.	238	0.051	0.791	n.s.
S.I.	239	0.215	3.395	< 0.001	S.I.	236	0.165	2.565	< 0.02
M.I.	242	0.161	2.519	< 0.02	M.I.	238	0.149	2.317	< 0.025
R.V.D.	233	0.230	3.588	< 0.001	R.V.D.	229	0.207	3.185	< 0.005

TABLE XLIII

The relation between x-ray indices and diet and diet divided by body weight in the combined normal and 'osteoporotic' groups of female subjects (n.s. - not significant).

TABLE XLIV

Fall in X-ray indices with age in male and female subjects.

MALES	No. of subjects	r	Significance	gradient	Intercept
M.I.	75	-0.19	n.s.	-	-
F.I.	75	-0.32	P < 0.01	-0.174	65.2
S.I.	75	0.13	n.s.	-	-
R.V.D.	71	-0.17	n.s.	-	-
FEMALES					
M.I.	242	-0.67	P < 0.001	-0.542	83.56
F.I.	242	-0.46	P < 0.001	-0.293	70.31
S.I.	239	-0.30	P < 0.001	-0.202	97.02
R.V.D.	233	-0.55	P < 0.001	-0.608	85.00

	Number of subjects	r	T-value	P		Number of subjects	r	T-value	P
Calcium					Calcium/body weight				
F.I.	68	0.247	2.071	< 0.05	F.I.	66	-0.014	-0.109	n.s.
S.I.	68	0.091	0.743	n.s.	S.I.	66	0.178	1.446	n.s.
M.I.	68	0.014	0.117	n.s.	M.I.	66	-0.006	-0.044	n.s.
R.V.D.	65	-0.186	-1.504	n.s.	R.V.D.	63	-0.267	-2.166	< 0.05
Phosphorus					Phosphorus/body weight				
F.I.	68	0.245	2.050	< 0.05	F.I.	66	-0.087	-0.702	n.s.
S.I.	68	0.126	1.033	n.s.	S.I.	66	0.228	1.872	n.s.
M.I.	68	-0.085	-0.096	n.s.	M.I.	66	-0.112	-0.898	n.s.
R.V.D.	65	-0.199	-1.609	n.s.	R.V.D.	63	-0.250	-2.021	< 0.05
Protein					Protein/body weight				
F.I.	68	0.272	2.300	< 0.025	F.I.	66	-0.081	-0.654	n.s.
S.I.	68	-0.123	-1.003	n.s.	S.I.	66	0.003	0.022	n.s.
M.I.	68	0.004	0.036	n.s.	M.I.	66	-0.027	-0.215	n.s.
R.V.D.	65	-0.144	-1.152	n.s.	R.V.D.	63	-0.193	-1.537	n.s.
Vitamin D					Vitamin D/body weight				
F.I.	68	0.108	0.879	n.s.	F.I.	66	-0.065	-0.525	n.s.
S.I.	68	0.097	0.794	n.s.	S.I.	66	0.122	0.986	n.s.
M.I.	68	0.016	0.134	n.s.	M.I.	66	0.009	0.072	n.s.
R.V.D.	65	0.042	0.333	n.s.	R.V.D.	63	0.023	0.183	n.s.

TABLE XLV

The relation between X-ray indices and diet and diet divided by body weight in normal male subjects (n.s. = not significant)

TABLE XLVI

The relation between dietary intake and age in normal male subjects.

Dietary Constituent	Number of subjects	r	T-value	P
Calcium	68	-0.194	-1.606	n.s.
Phosphorus	68	-0.185	-1.531	n.s.
Protein	68	-0.267	-2.249	< 0.05
Vitamin D	68	0.019	0.158	n.s.
Calcium/body weight	66	-0.049	-0.392	n.s.
Phosphorus/ body weight	66	-0.010	-0.078	n.s.
Protein/body weight	66	-0.079	-0.636	n.s.
Vitamin D/body weight	66	0.109	0.880	n.s.

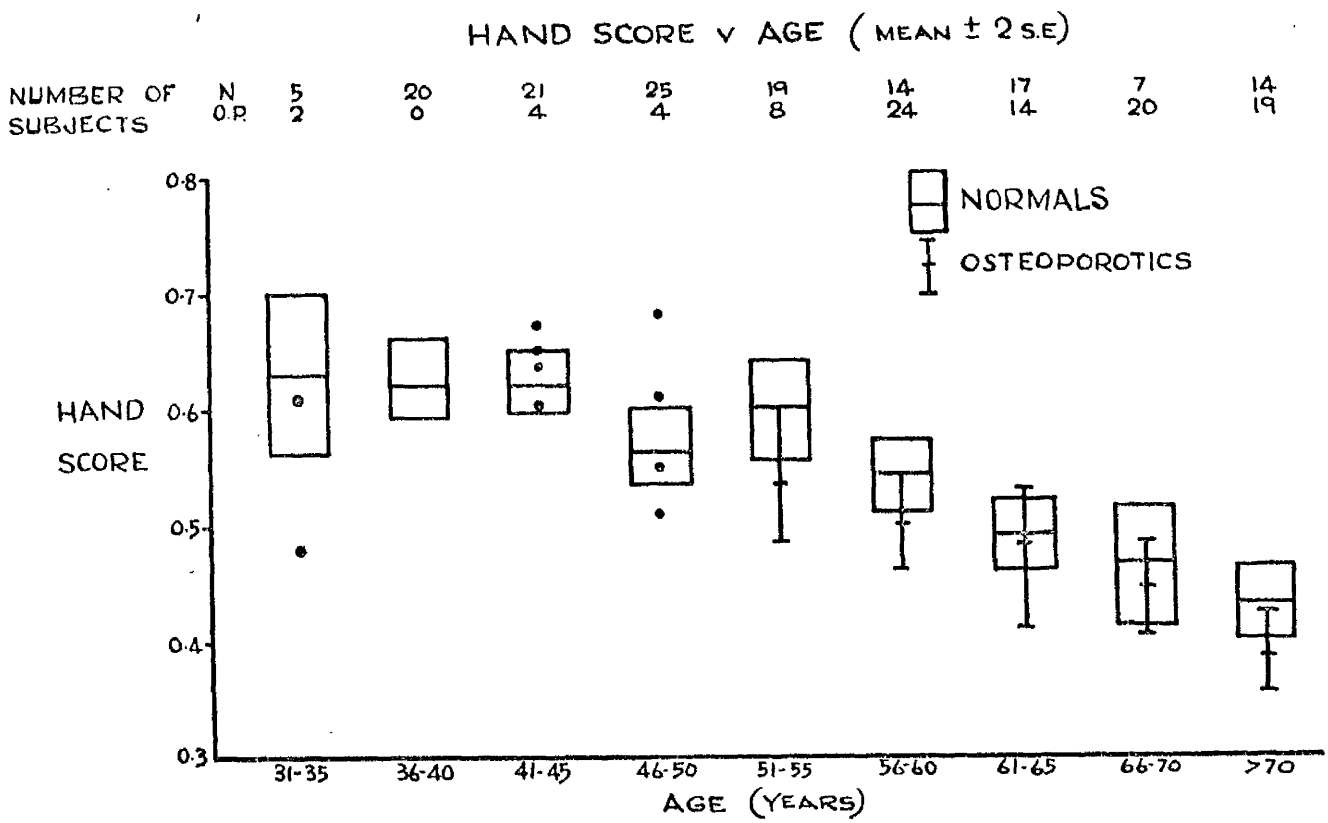


Fig 85 The Metacarpal Index plotted against age in "osteoporotic" and normal female subjects. At no decade is there any significant difference between the two groups. The mean and 2 S.E. range in 5 year age groups are shown.

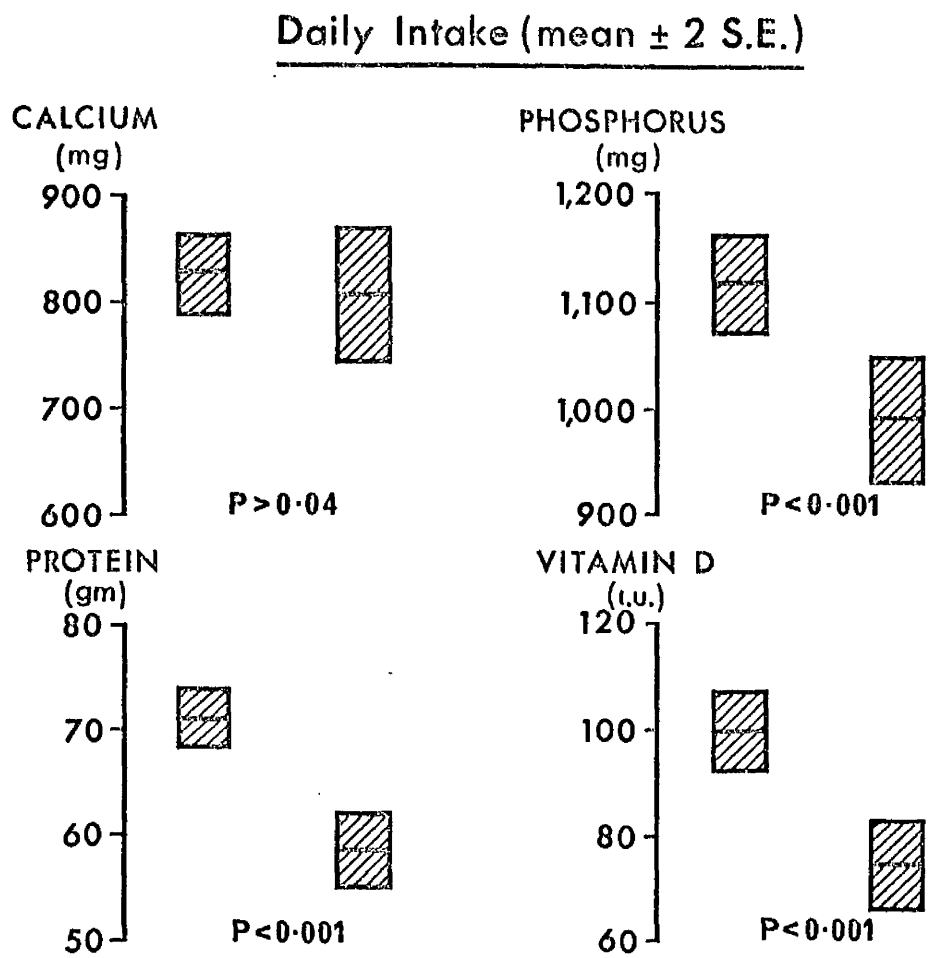


Fig 86 Dietary intake of calcium, phosphorus, protein and vitamin D in normal and "osteoporotic" subjects. The mean and 2 S.E. range are shown. The "osteoporotic" group has a significantly lower intake of phosphorus, protein and vitamin D.

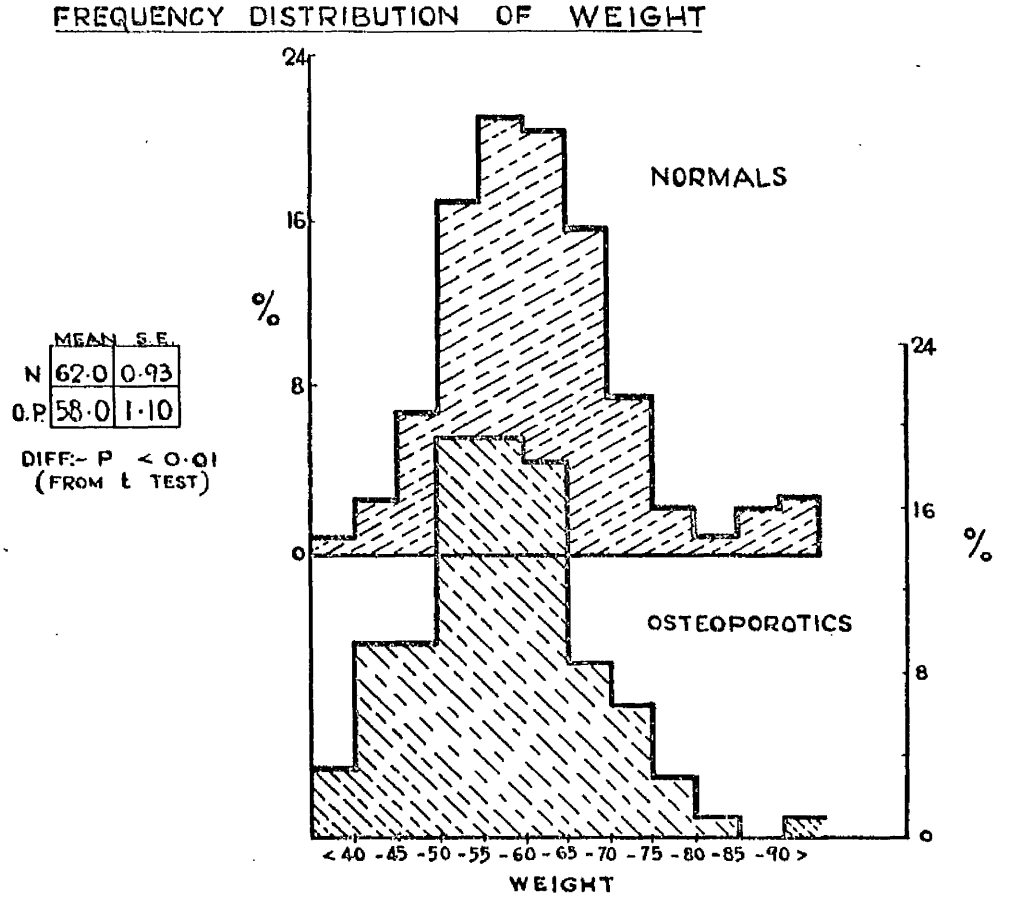
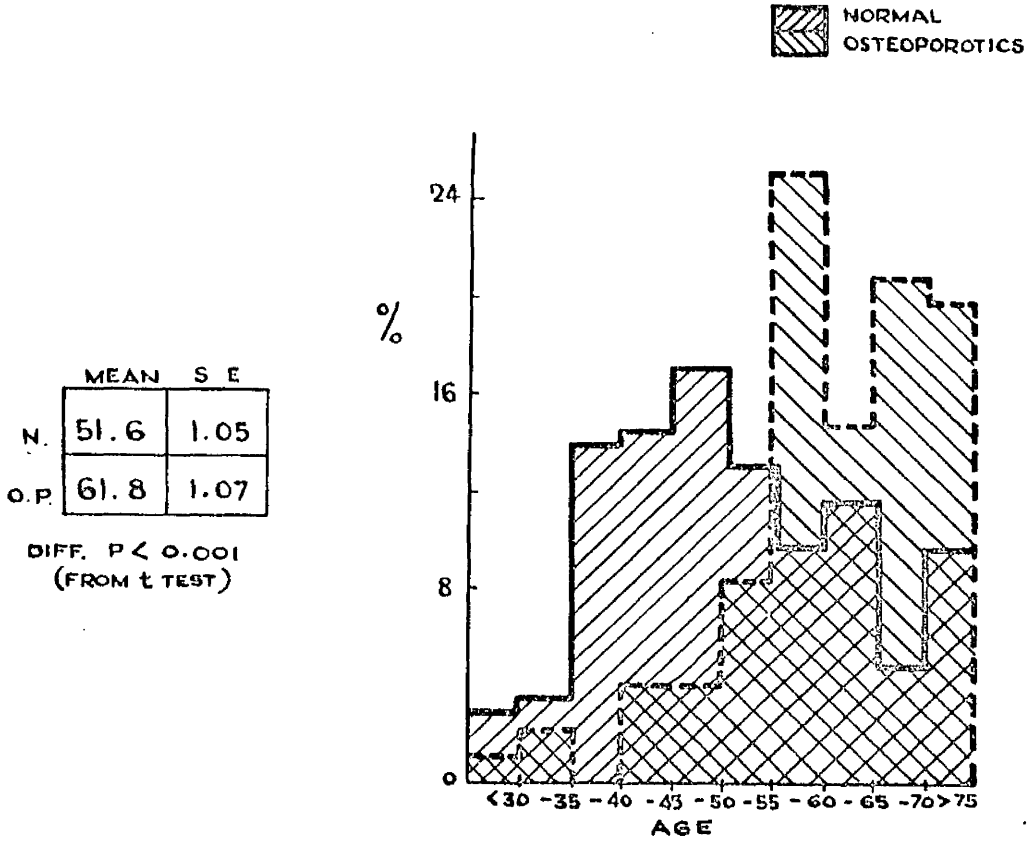


Fig 87 The frequency distribution of body weight in the normal and "osteoporotic" subjects in 5 year age groups. The "osteoporotic" group is significantly lighter than the normal subjects.

FREQUENCY DISTRIBUTION OF AGE



	MEAN	S E
N.	51.6	1.05
O.P.	61.8	1.07

DIFF. $P < 0.001$
(FROM t TEST)

Fig 88 The frequency distribution of age in the normal and "osteoporotic" subjects in 5 year age groups. The "osteoporotic" group is significantly older than the normal group.

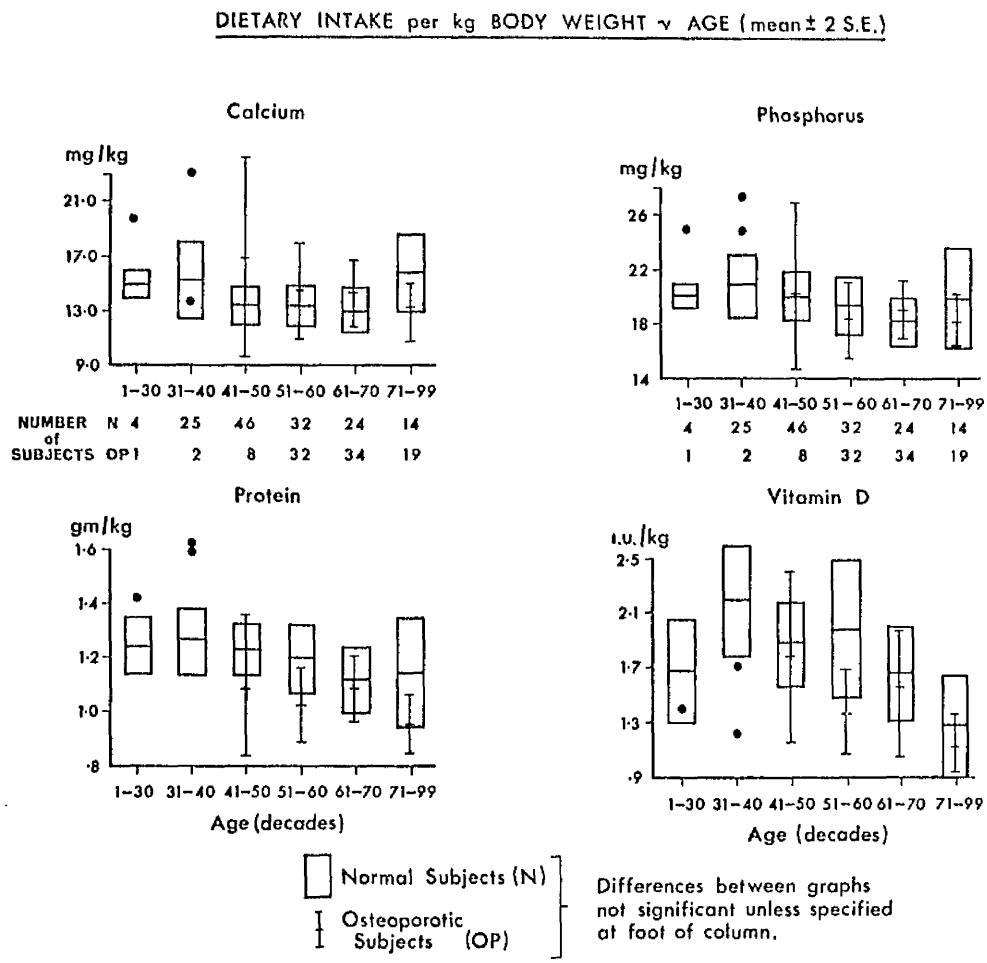


Fig 89 Dietary intake in the normal and "osteoporotic" subjects divided by body weight and plotted against age in 10-year age groups. The mean and 2 S.E. range are shown. No significant difference between the groups is found.

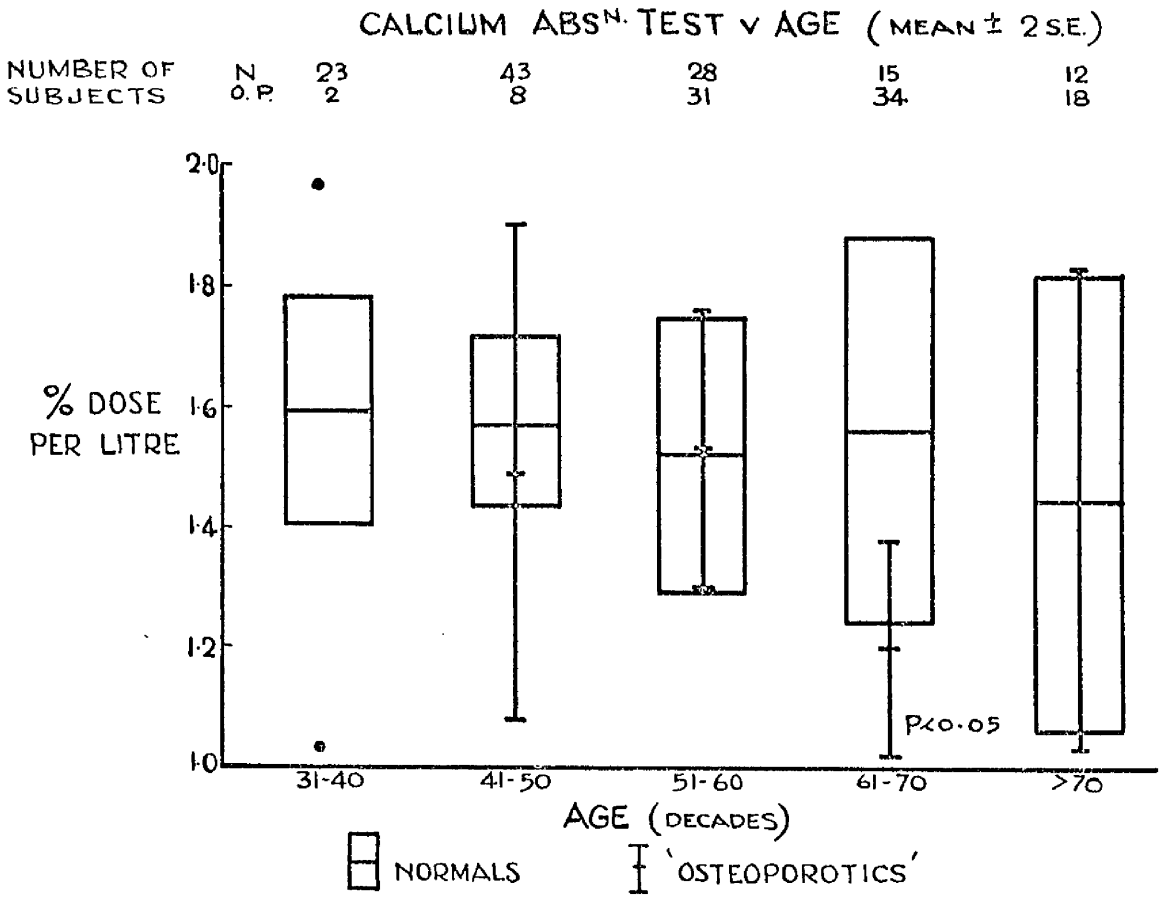


Fig 90 Calcium absorption measured with 45Ca in the normal and "osteoporotic" subjects. The mean and 2 S.E. range in 10 year age groups are plotted against age. The two groups do not differ, except between 61 and 70 years of age.

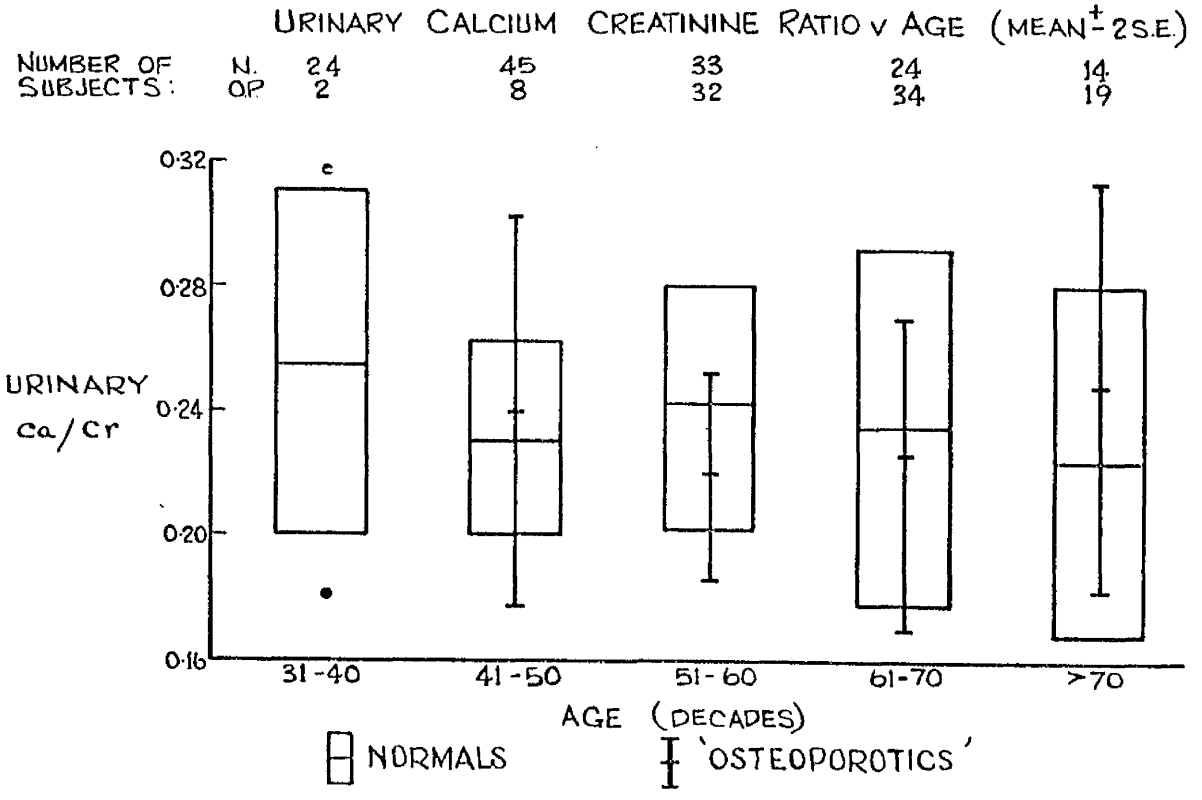


Fig 91 The mean and 2 S.E. range of two estimations of the urinary calcium:creatinine ratio plotted against age in 10 year age groups in the normal and "osteoporotic" subjects. No significant difference between the groups is seen.

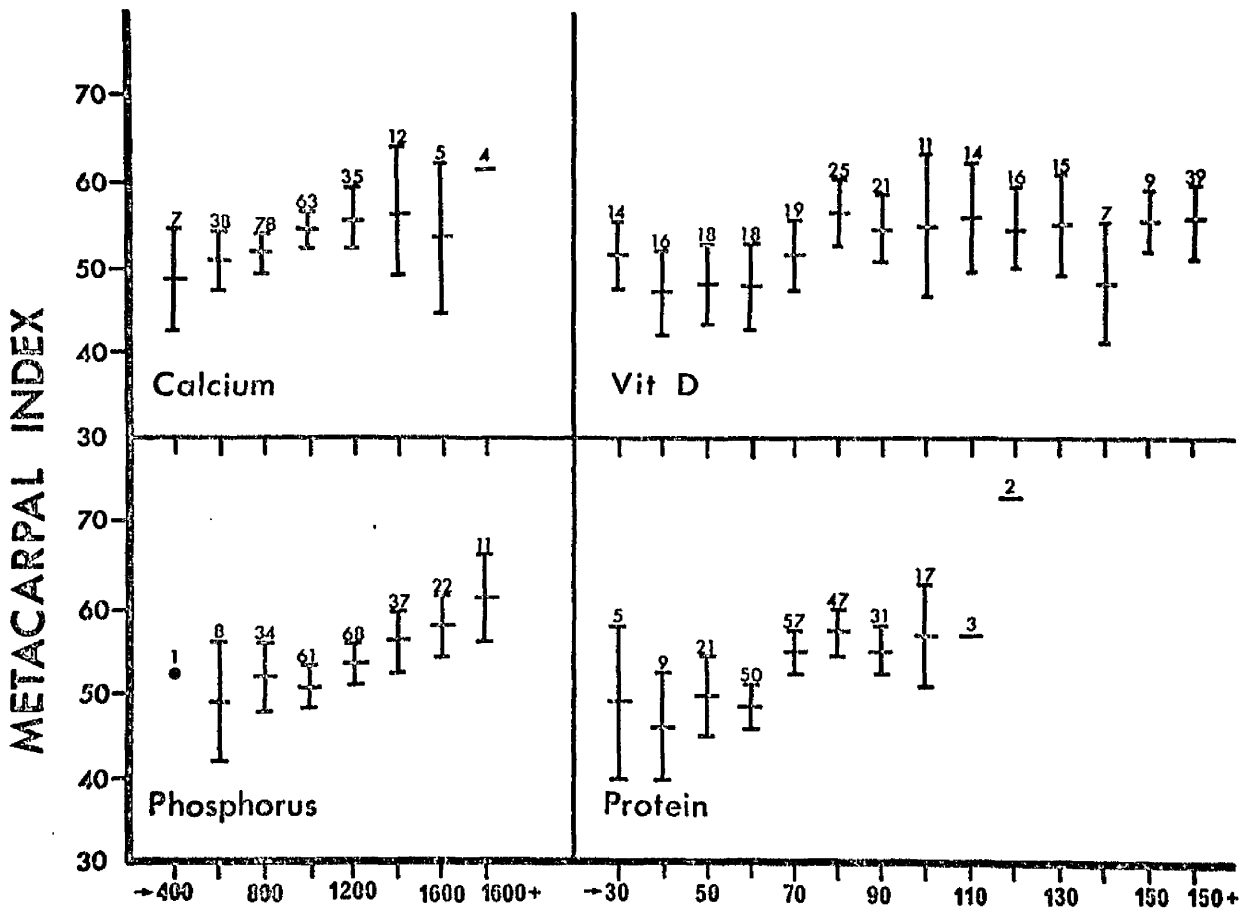


Fig 92 The relation between the Metacarpal Index and the dietary intake of calcium, phosphorus, protein and vitamin D in women. The mean and 2 S.E. range are shown. (The significance of the correlations are shown in Table XLIII).

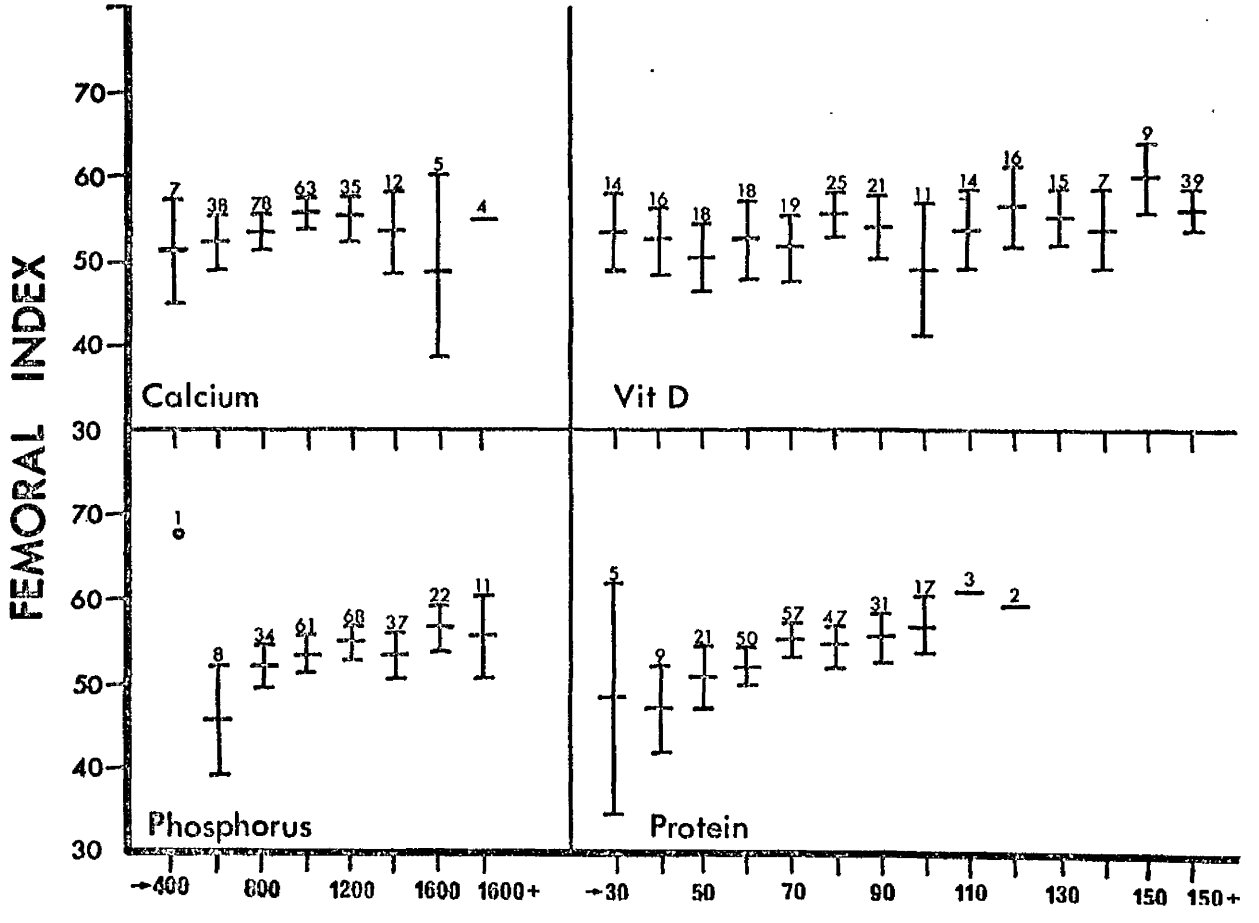


Fig 93 The relation between the Femoral Index and the dietary intake of calcium, phosphorus, protein and vitamin D in women. The mean and 2 S.E. range are shown. (The significance of the relation between F.I. and age are shown in Table XLIII).

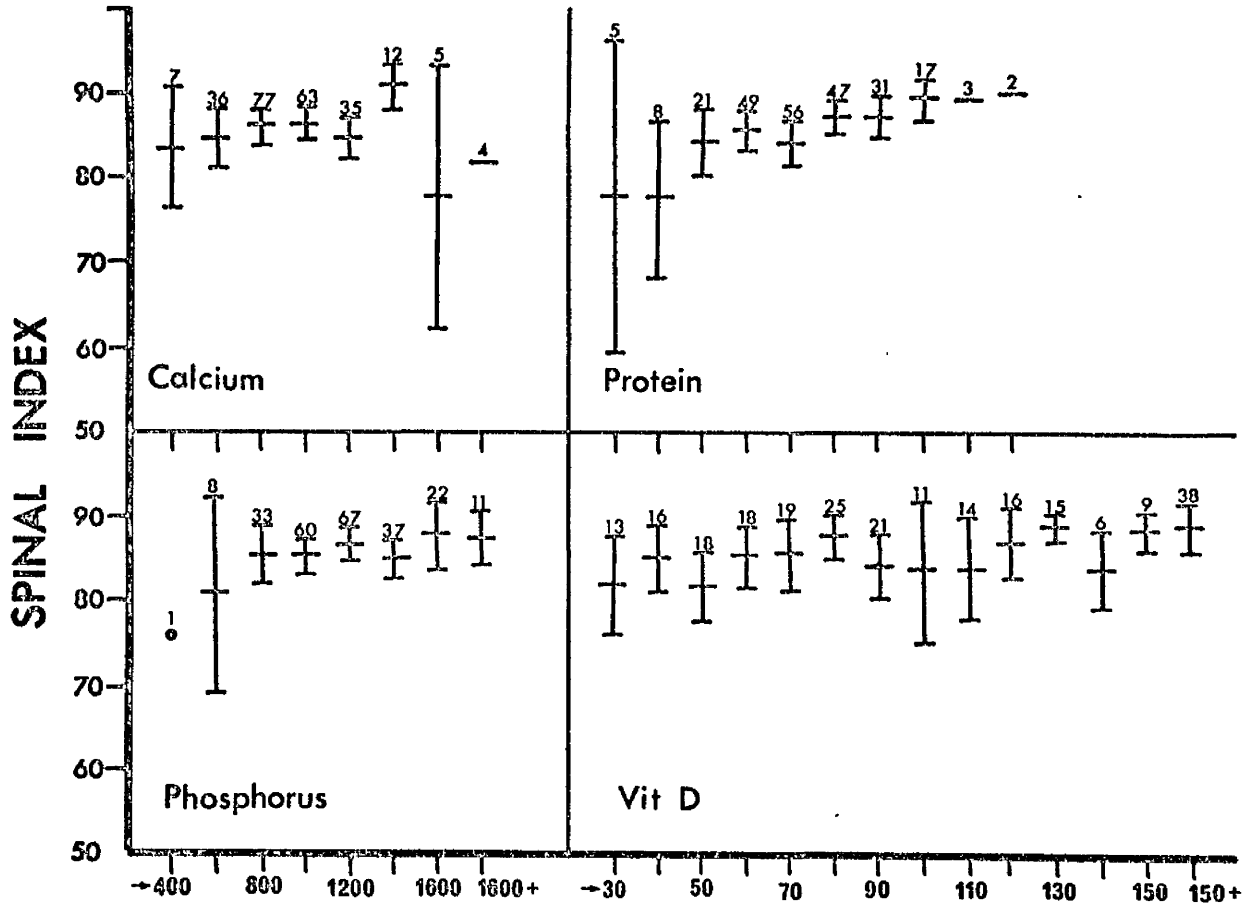


Fig 94 The relation between the Spinal Index and dietary intake of calcium, phosphorus, protein and vitamin D in women. The mean and 2 S.E. range are shown. (see Table XLIII).

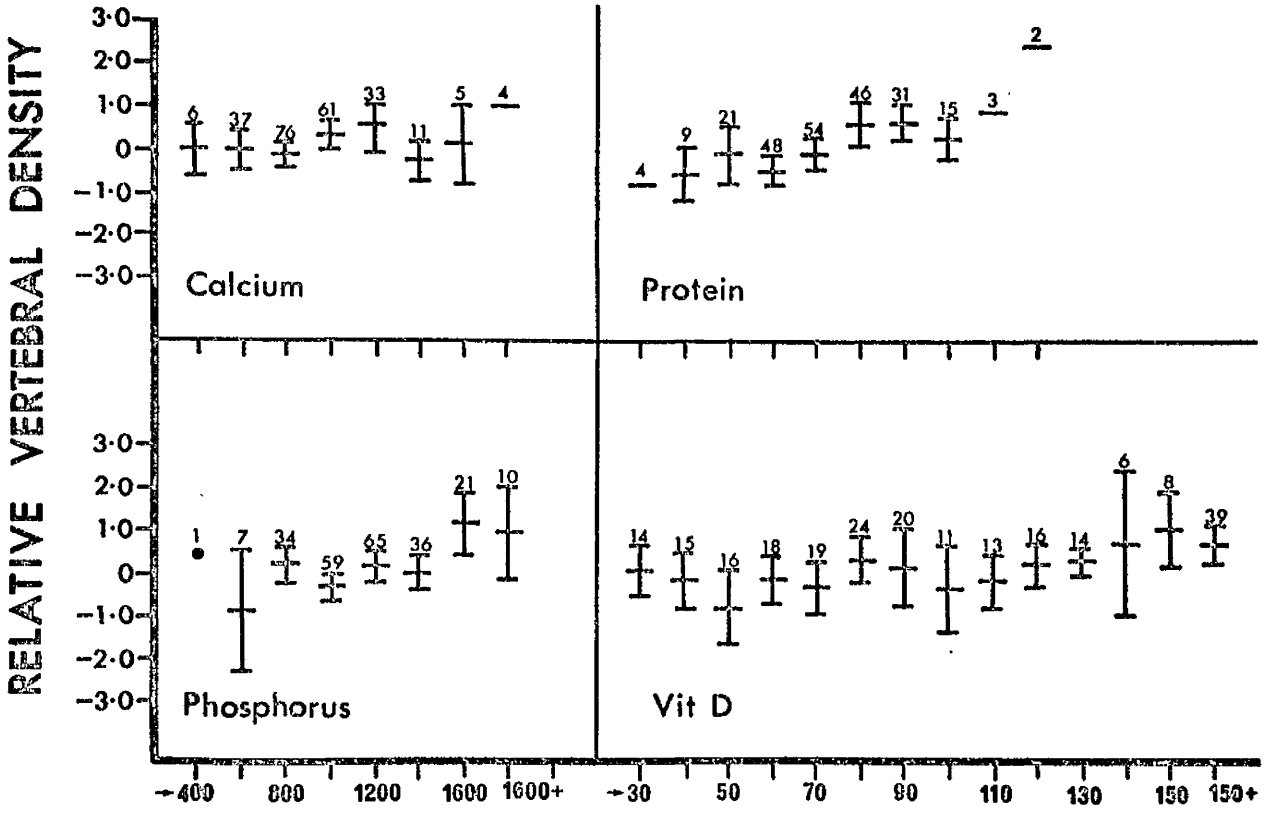


Fig 95 The relation between the Relative Vertebral Density and dietary intake of calcium, phosphorus, protein and vitamin D in women. The mean and 2 S.E. range are shown (see Table XLIII).

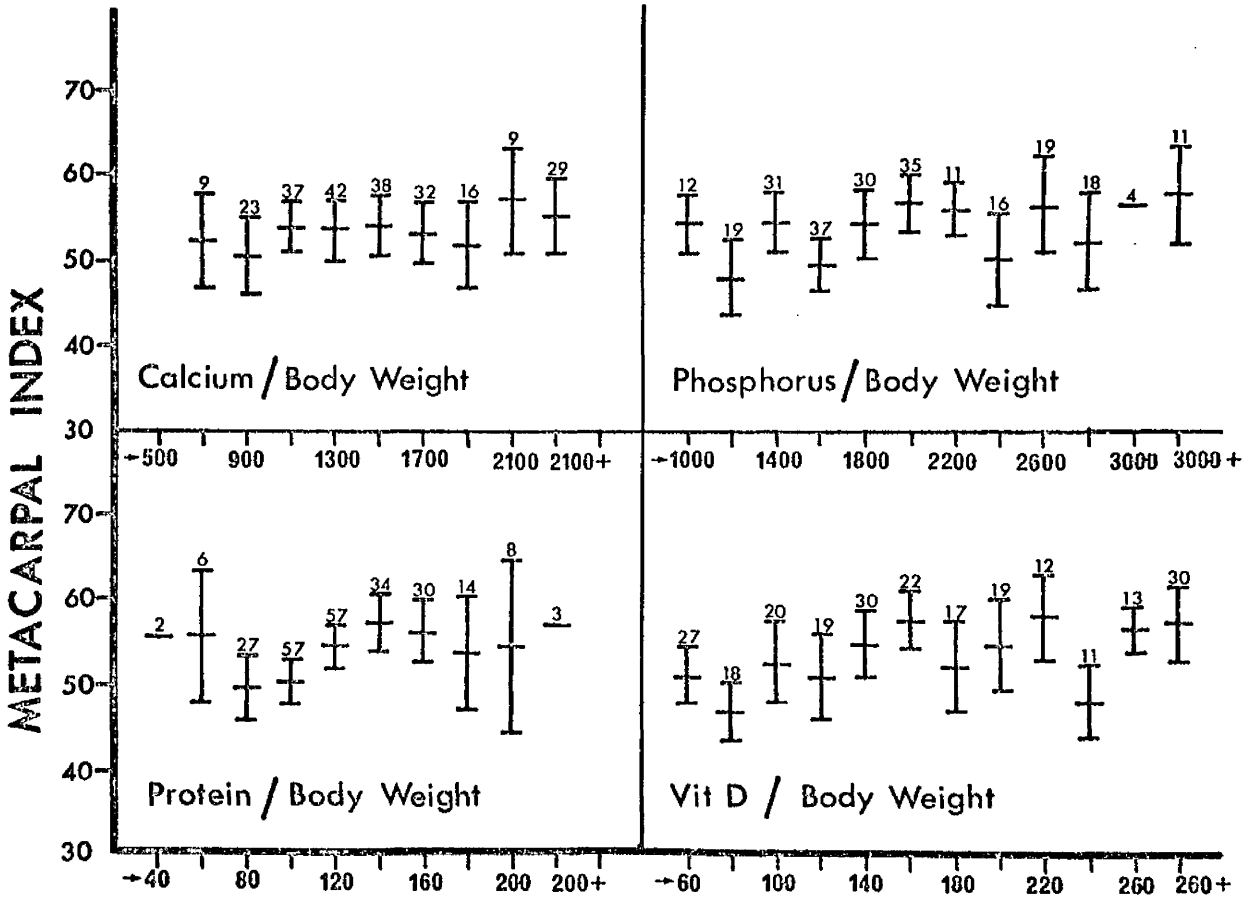


Fig 96 The relation between the Metacarpal Index and dietary intake divided by body weight in women. The mean and 2 S.E. range are shown (see Table XLIII).

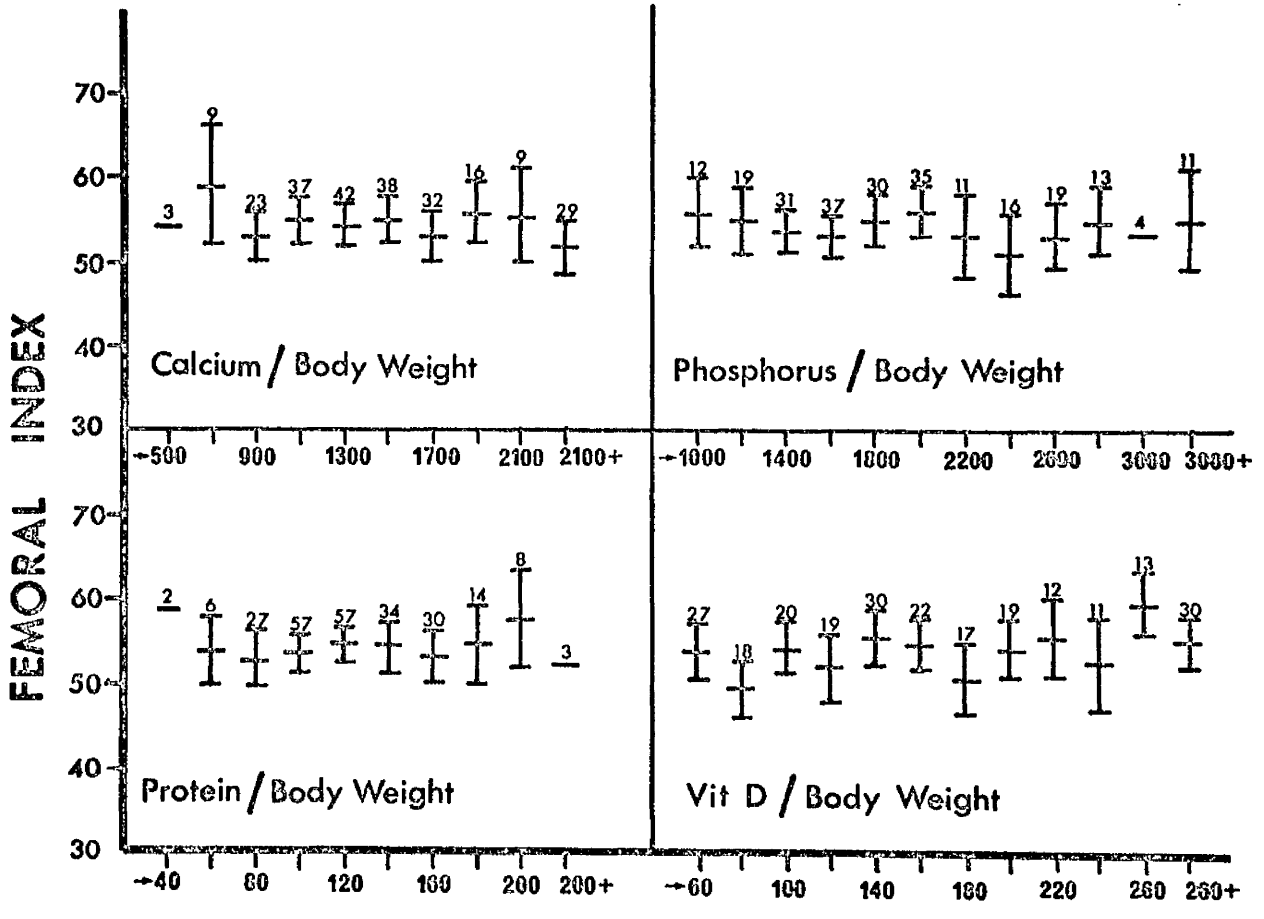


Fig 97 The relation between the Femoral Index and dietary intake divided by body weight in women. The mean and 2 S.E. range are shown (see Table XLIII).

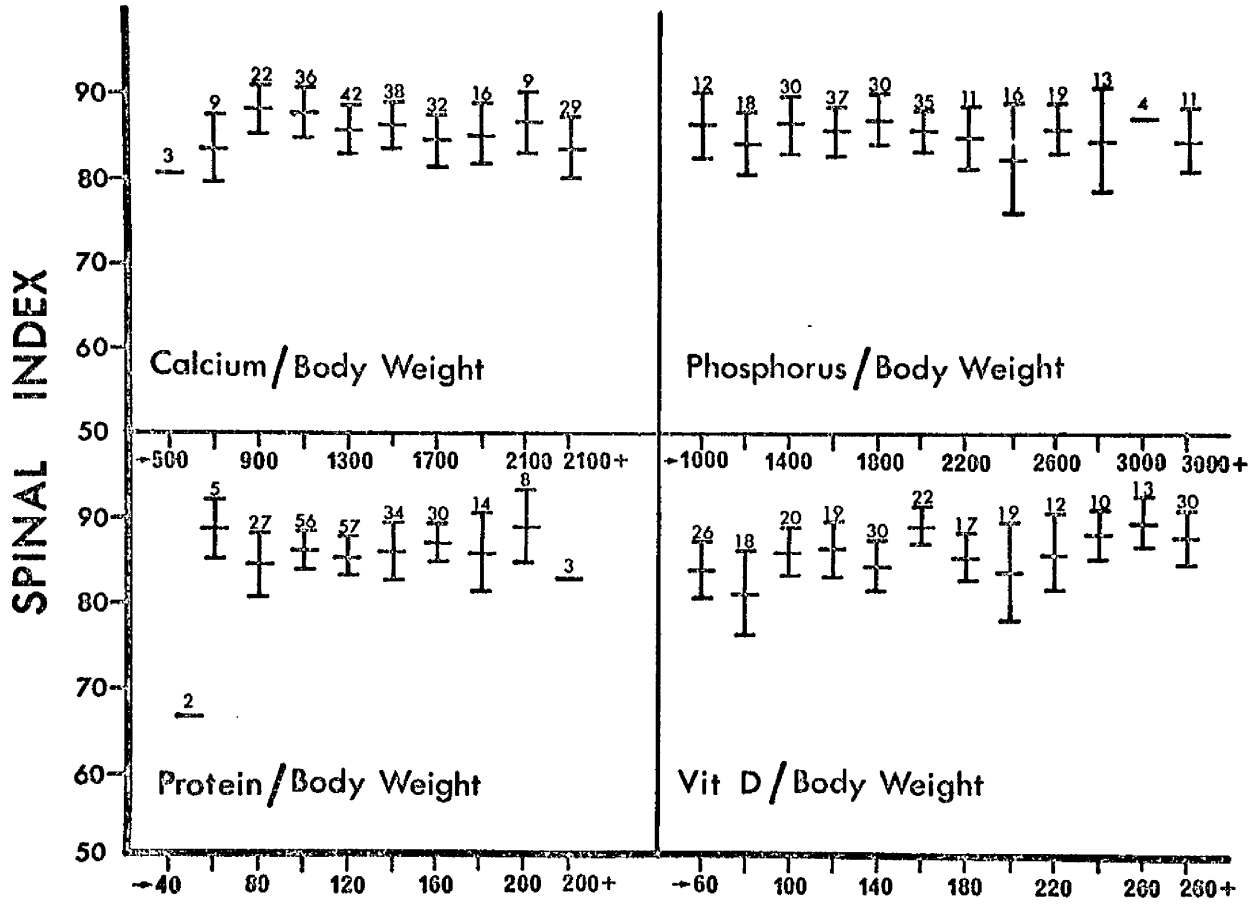


Fig 98 The relation between the Spinal Index and dietary intake divided by body weight in women. The mean and 2 S.E. range are shown (see Table XLIII).

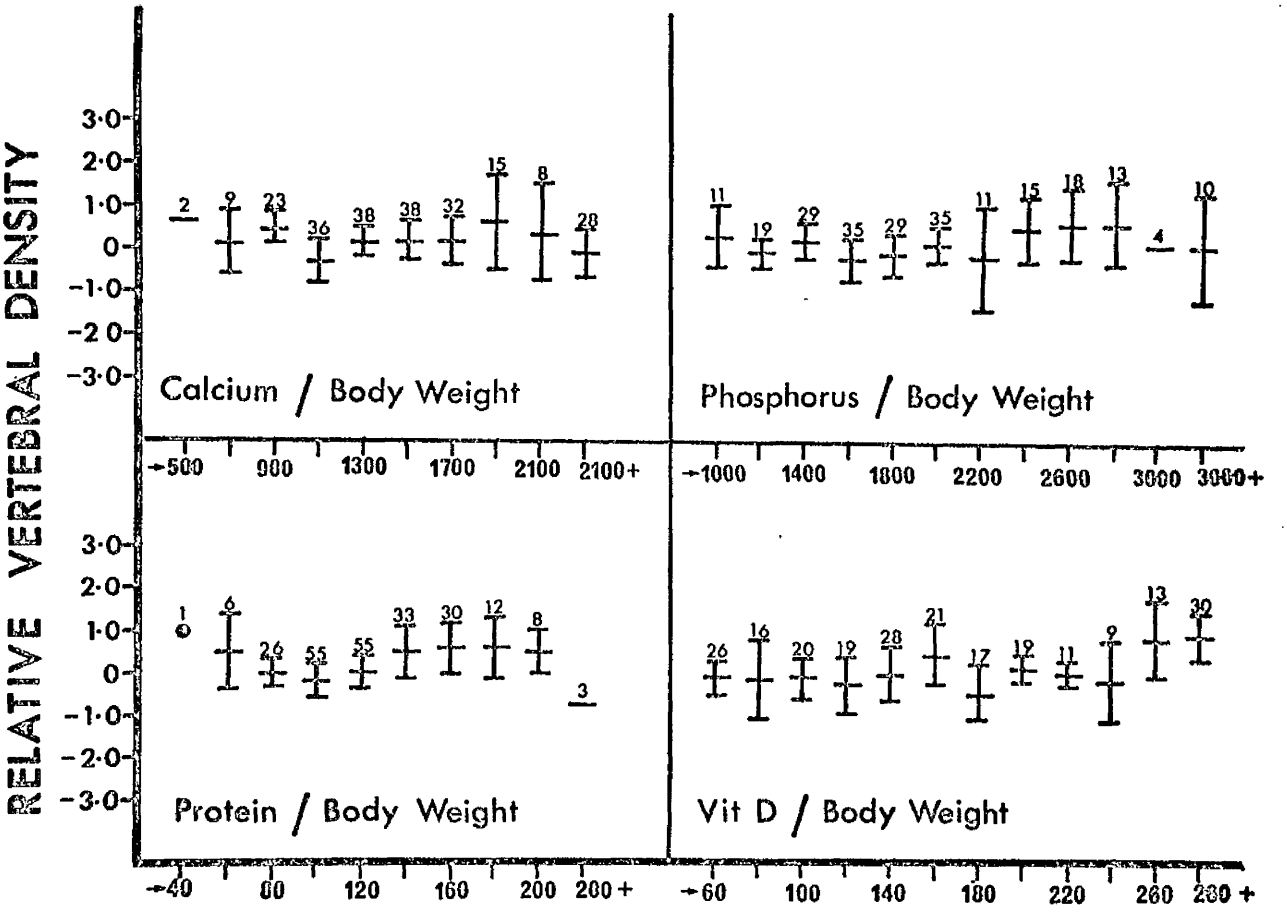


Fig 99 The relation between the Relative Vertebral Density and dietary intake, divided by body weight, in women. The mean and 2 S.E. range are shown (see Table XLIII).

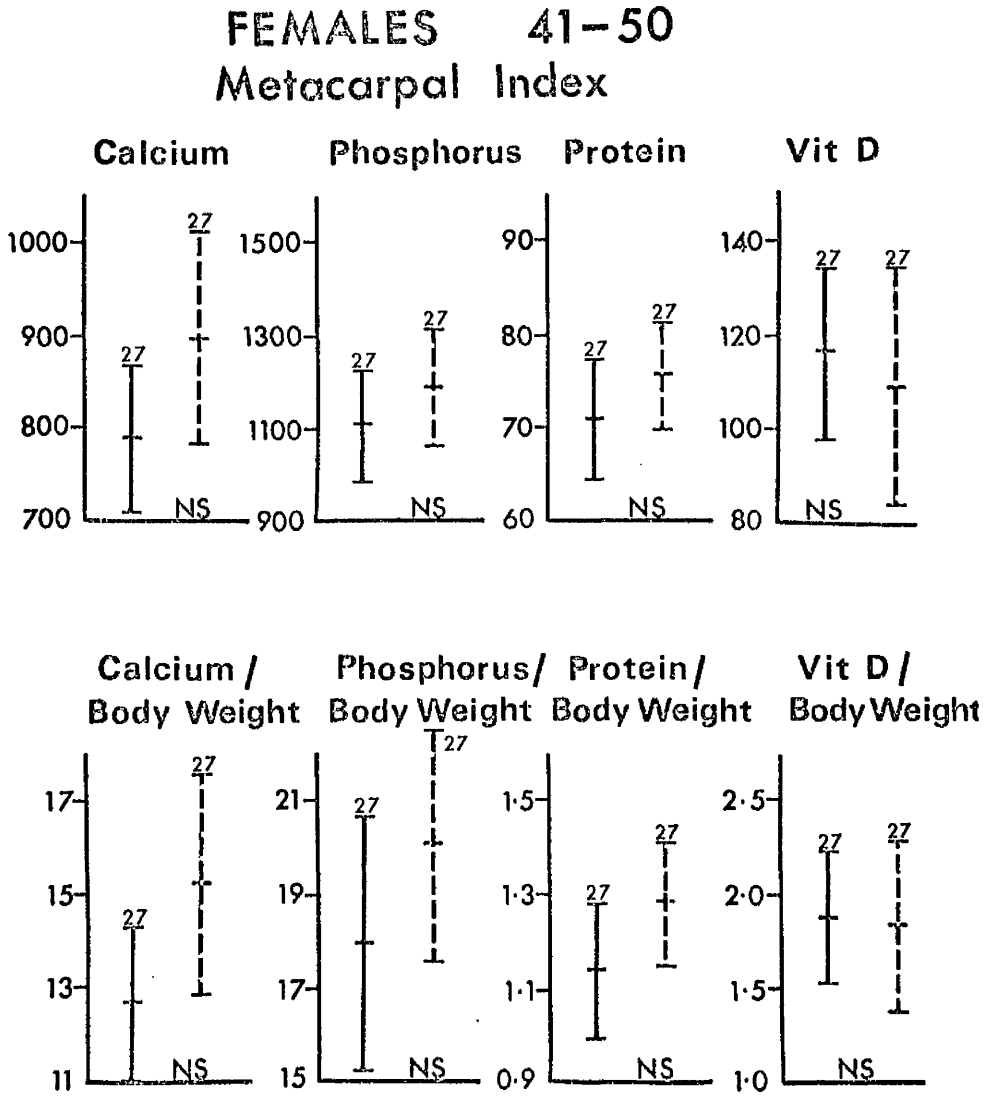


Fig 100 The dietary intake in women between 41 and 50 years of age with Metacarpal Indices above and below the mean value of the index. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean M.I. and the interrupted line shows those above the mean M.I.

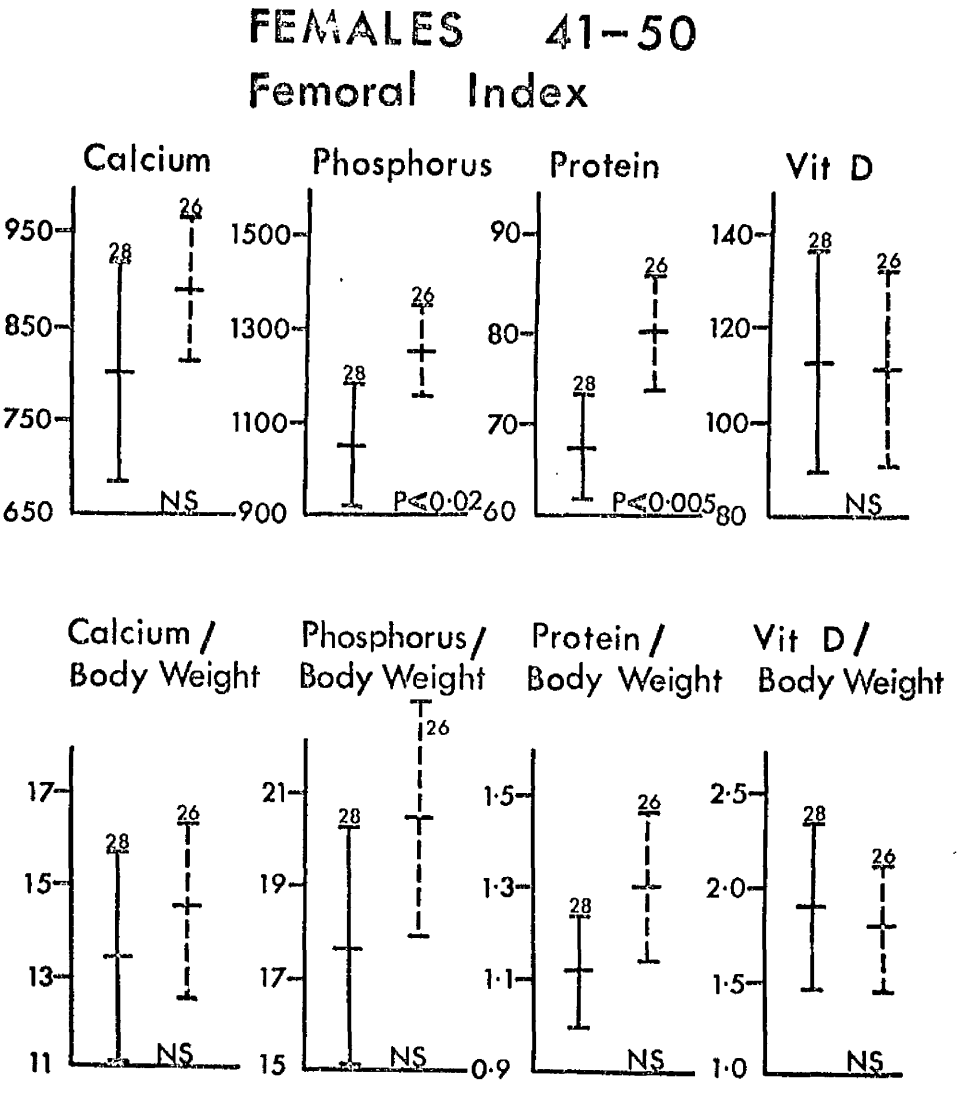


Fig 101 The dietary intake in women between 41 and 50 years of age with Femoral Indices above and below the mean value of the index. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean F.I. and the interrupted line shows those above the mean F.I.

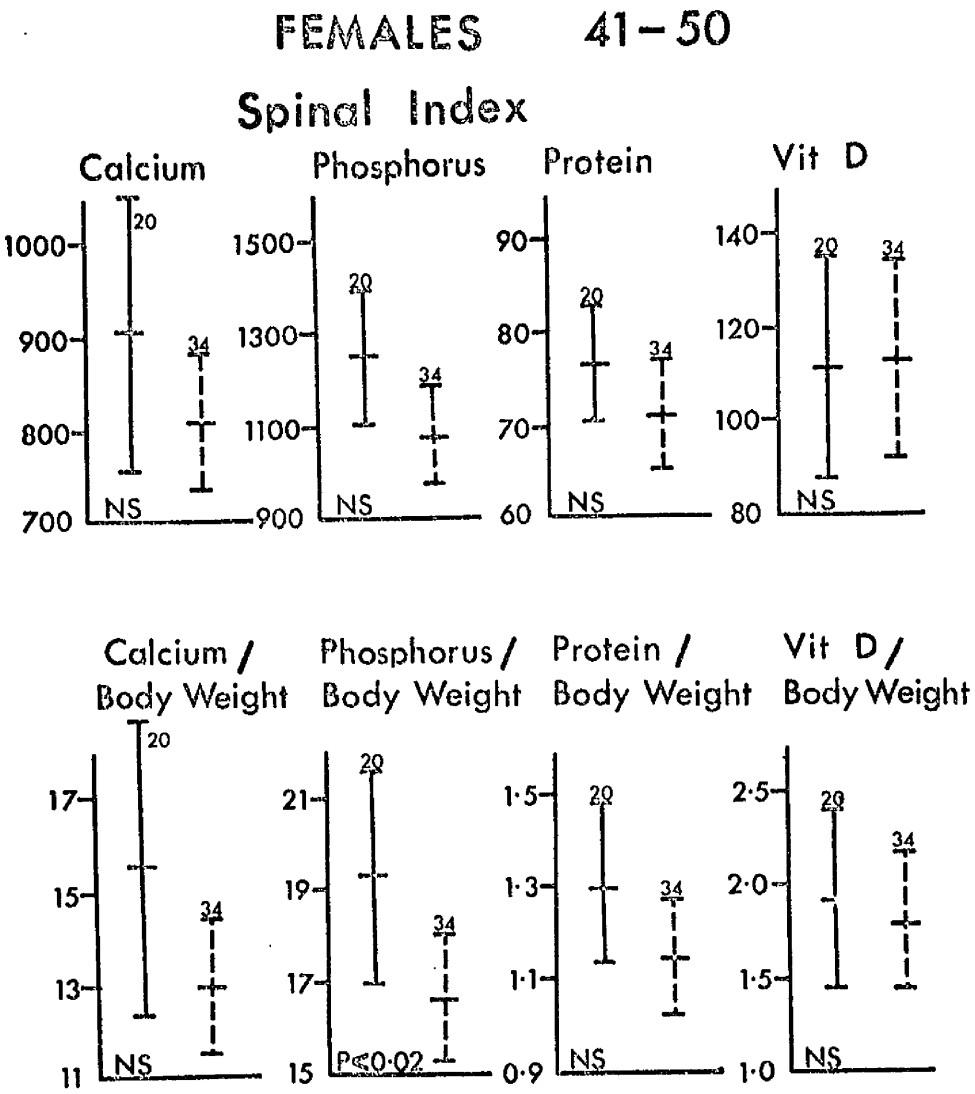


Fig 102 The dietary intake in women between 41 and 50 years of age with Spinal Indices above and below the mean value of the index. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean S.I. and the interrupted line shows those above the mean S.I.

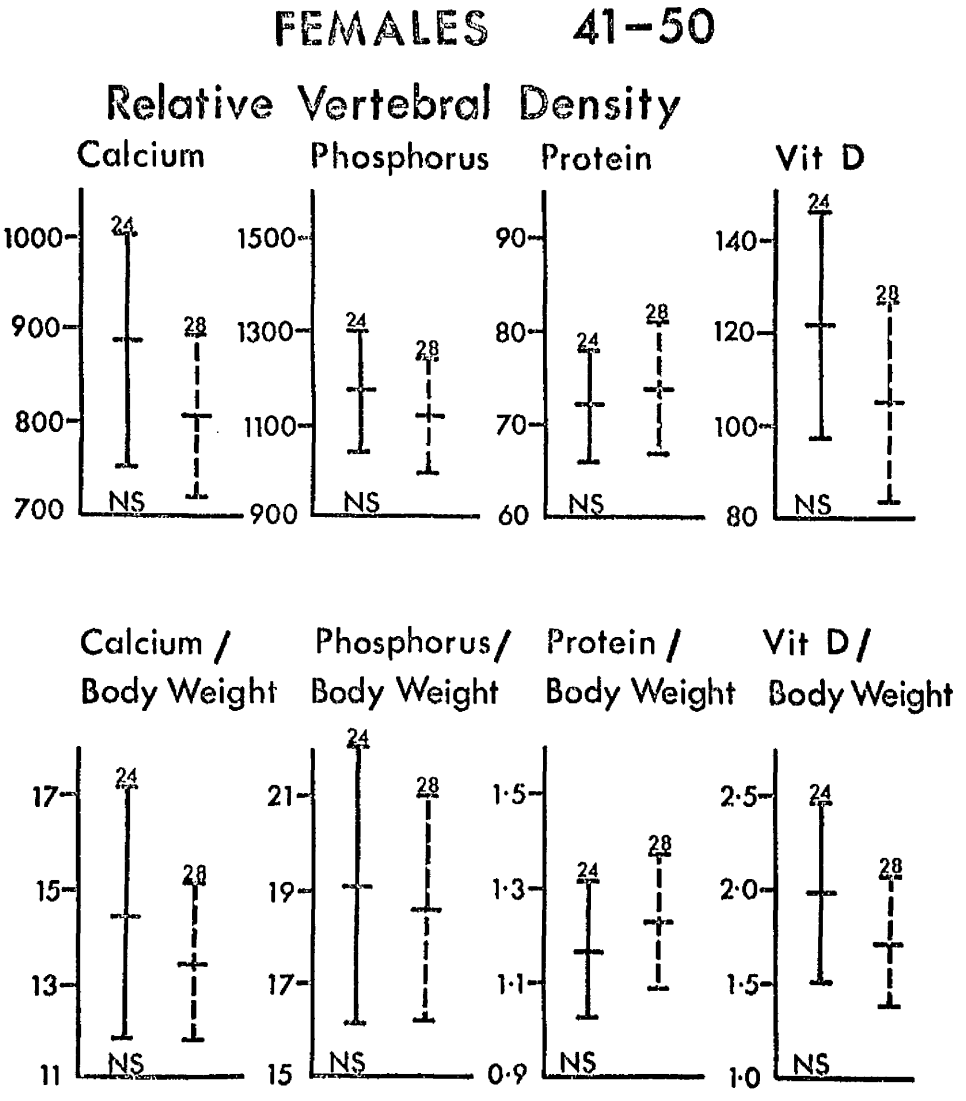


Fig 103 The dietary intake in women between 41 and 50 years of age with Relative Vertebral Densities above and below the mean value of the R.V.D. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean R.V.D. and the interrupted line shows those above the mean R.V.D.

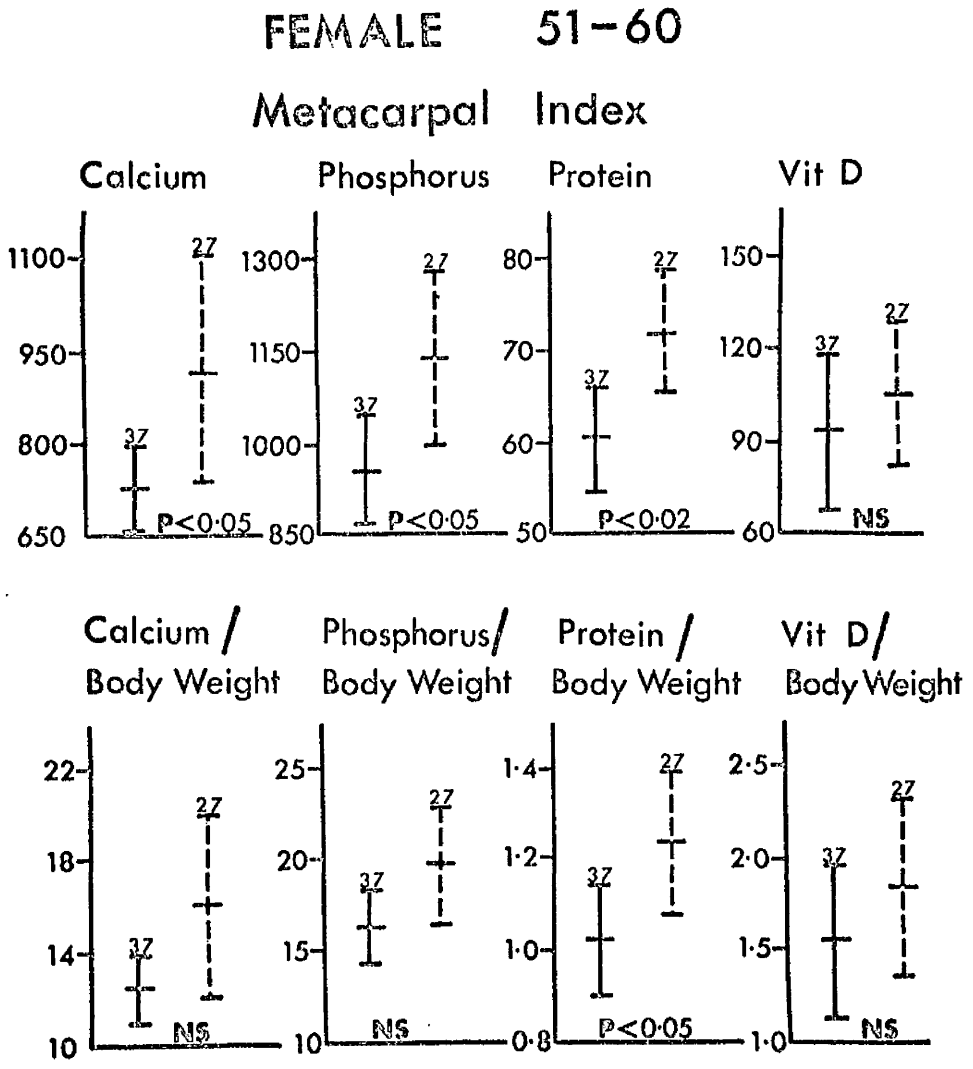


Fig 104 The dietary intake in women between 51 and 60 years of age with Metacarpal Indices above and below the mean value of the index. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean M.I. and the interrupted line shows those above the mean M.I.

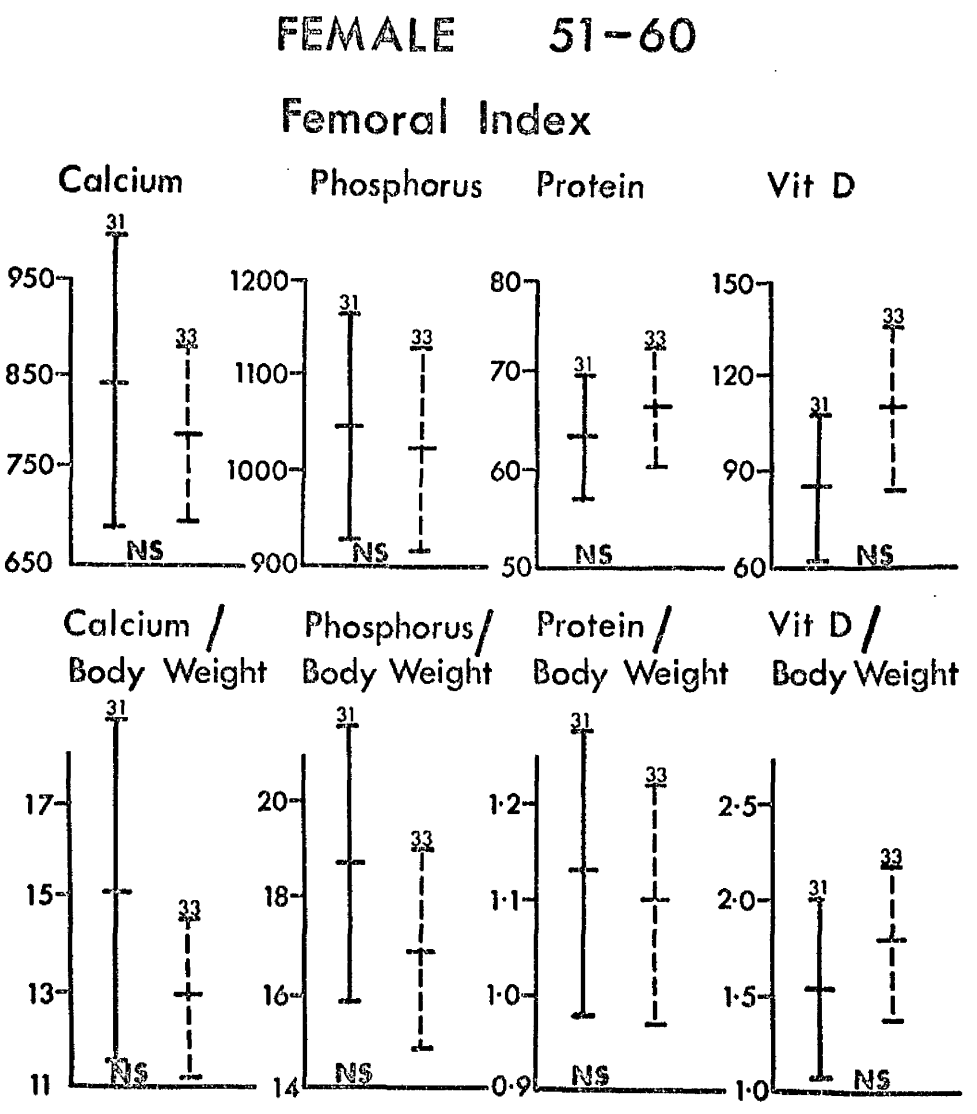


Fig 105 The dietary intake in women between 51 and 60 years of age with Femoral Indices above and below the mean value of the index. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean F.I. and the interrupted line shows those above the mean F.I.

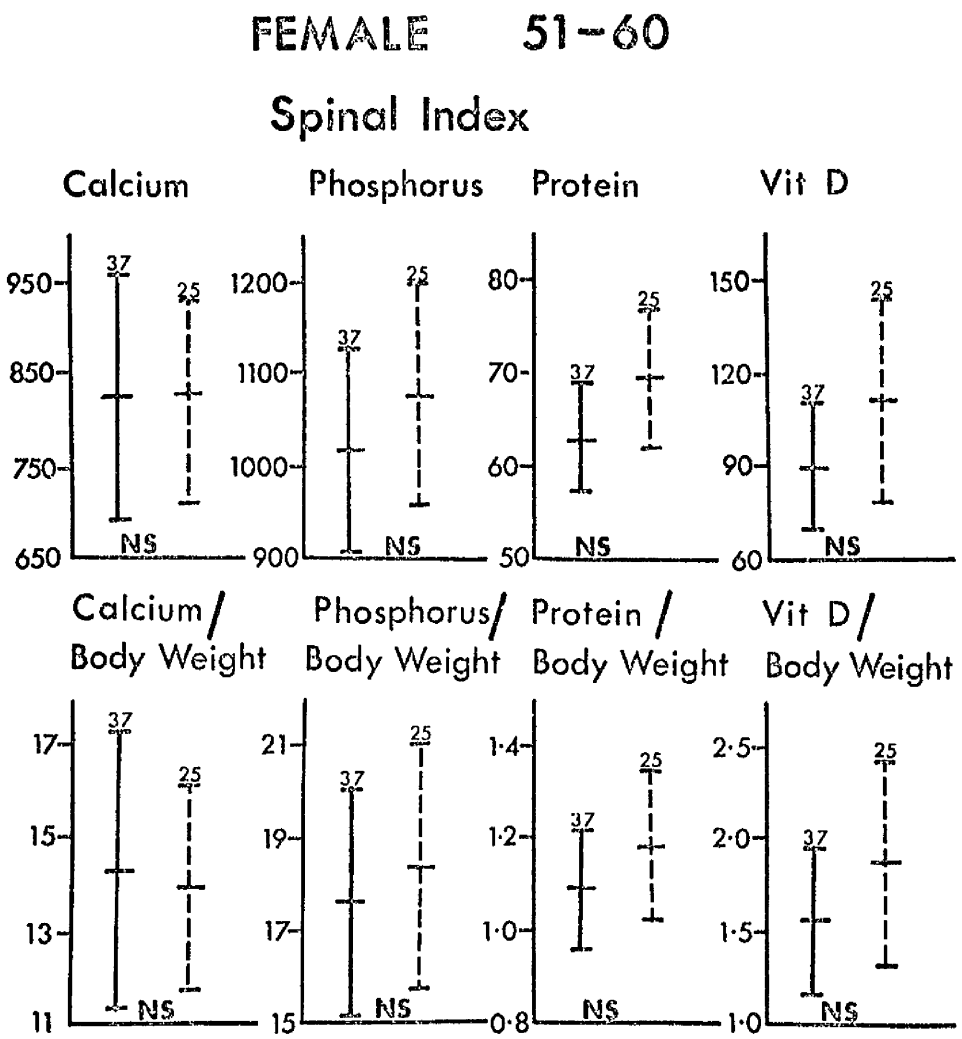


Fig 106 The dietary intake in women between 51 and 60 years of age with Spinal Indices above and below the mean value of the index. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean S.I. and the interrupted line shows those above the mean S.I.

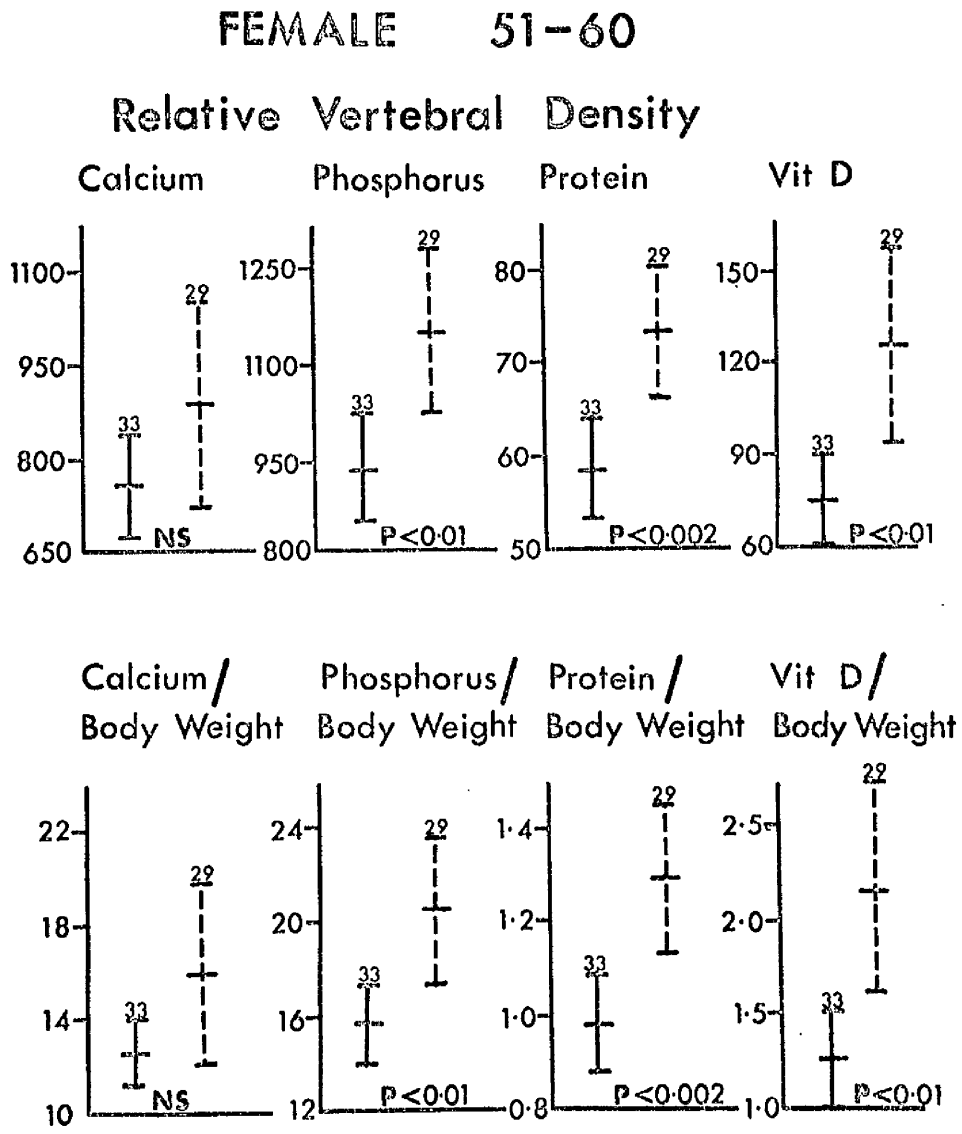


Fig 107 The dietary intake in women between 51 and 60 years of age with Relative Vertebral Densities above and below the mean R.V.D. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean R.V.D. and the interrupted line shows those above the mean R.V.D.

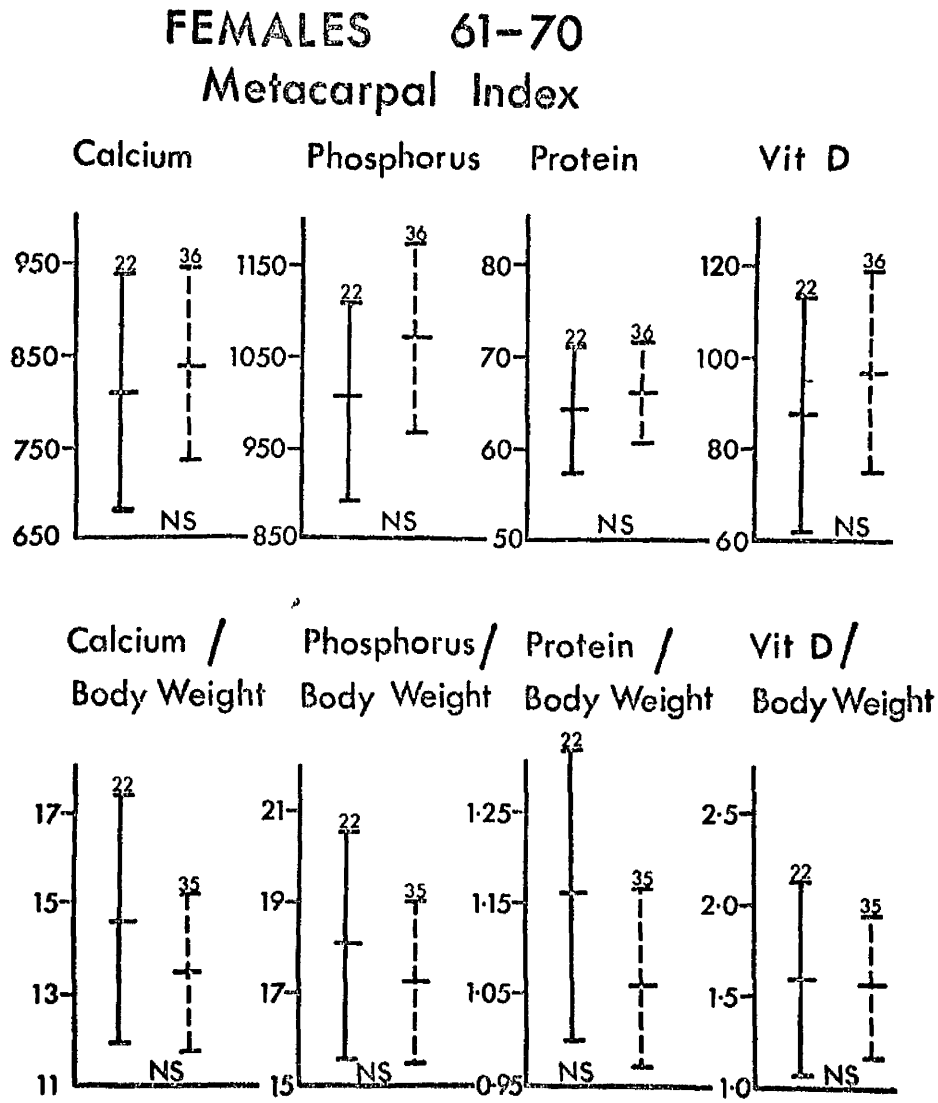


Fig 108 The dietary intake in women between 61 and 70 years of age with Metacarpal Indices above and below the mean value of the index. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean M.I. and the interrupted line shows those above the mean M.I.

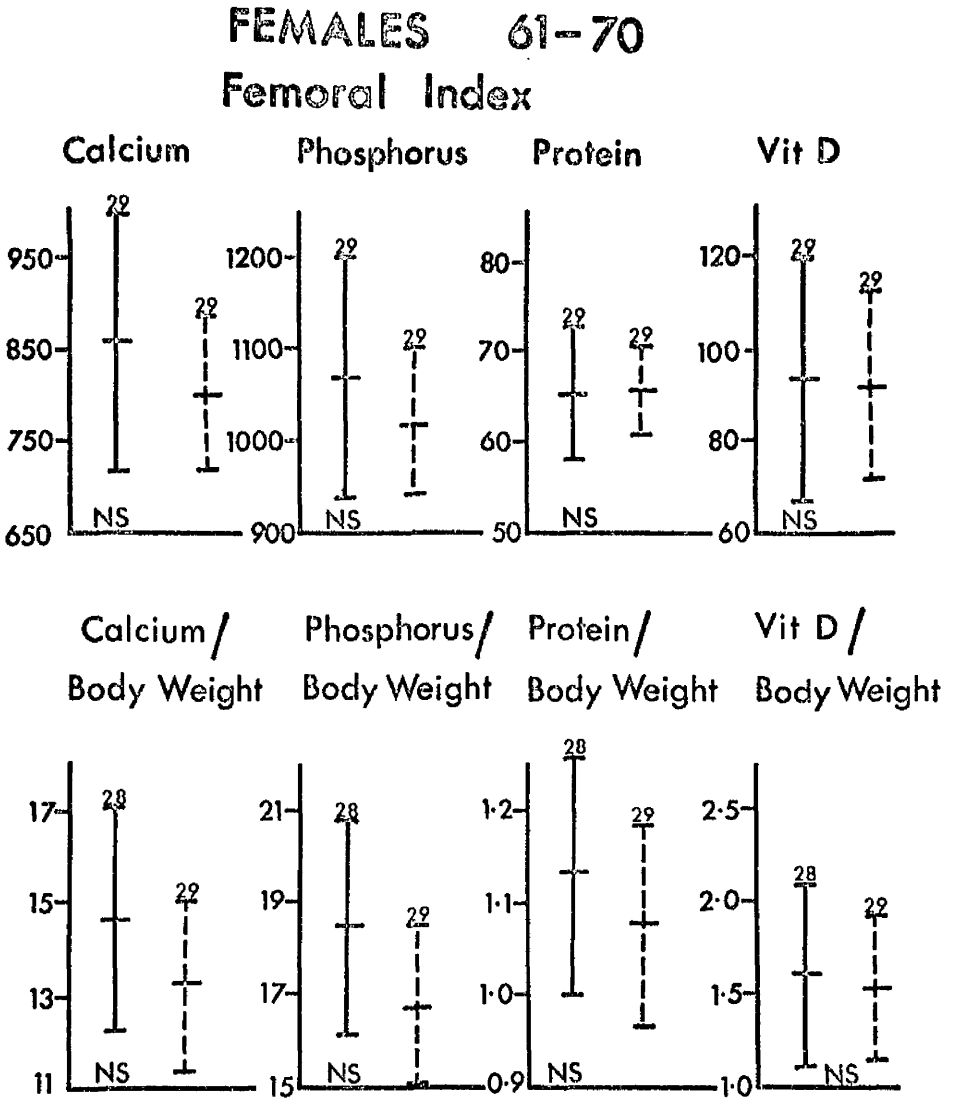


Fig 109 The dietary intake in women between 61 and 70 years of age with Femoral Indices above and below the mean value of the index. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean F.I. and the interrupted line shows those above the mean F.I.

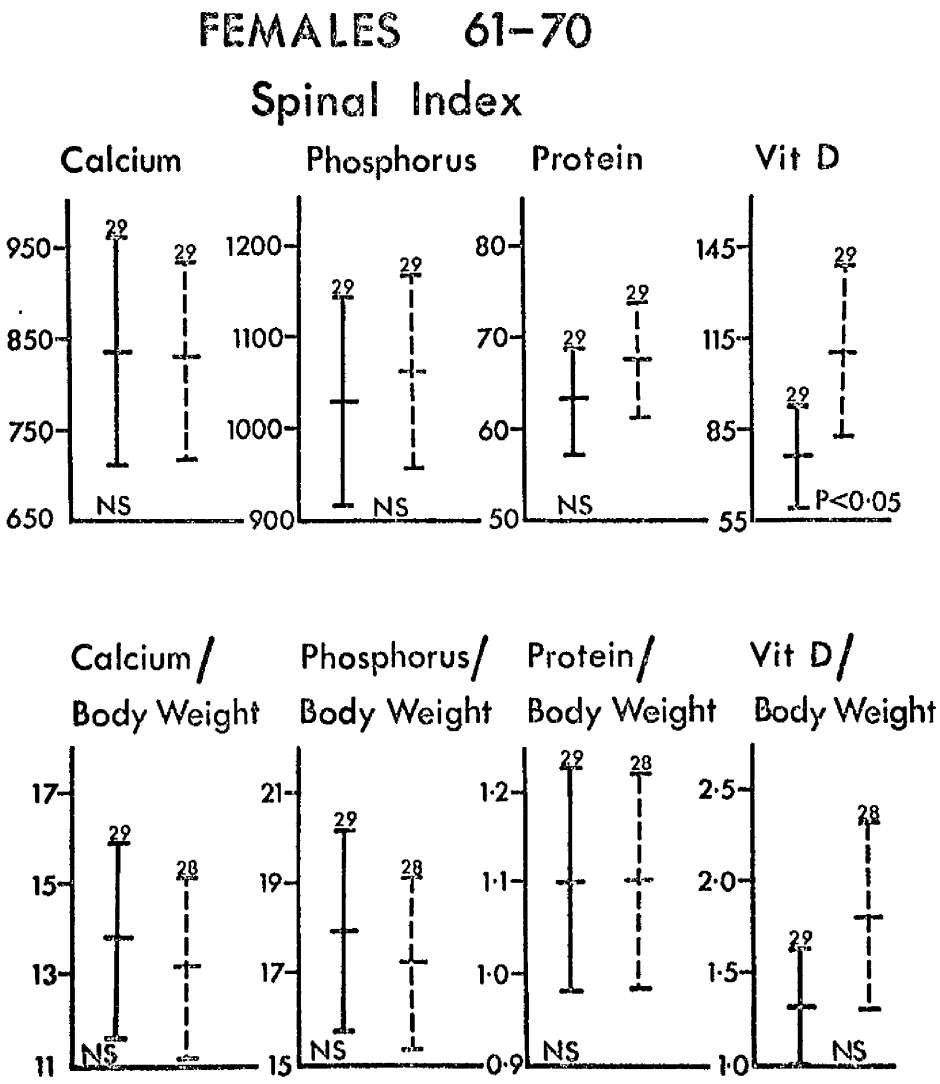


Fig 110 The dietary intake in women between 61 and 70 years of age with Spinal Indices above and below the mean value of the index. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean S.I. and the interrupted line shows those above the mean S.I.

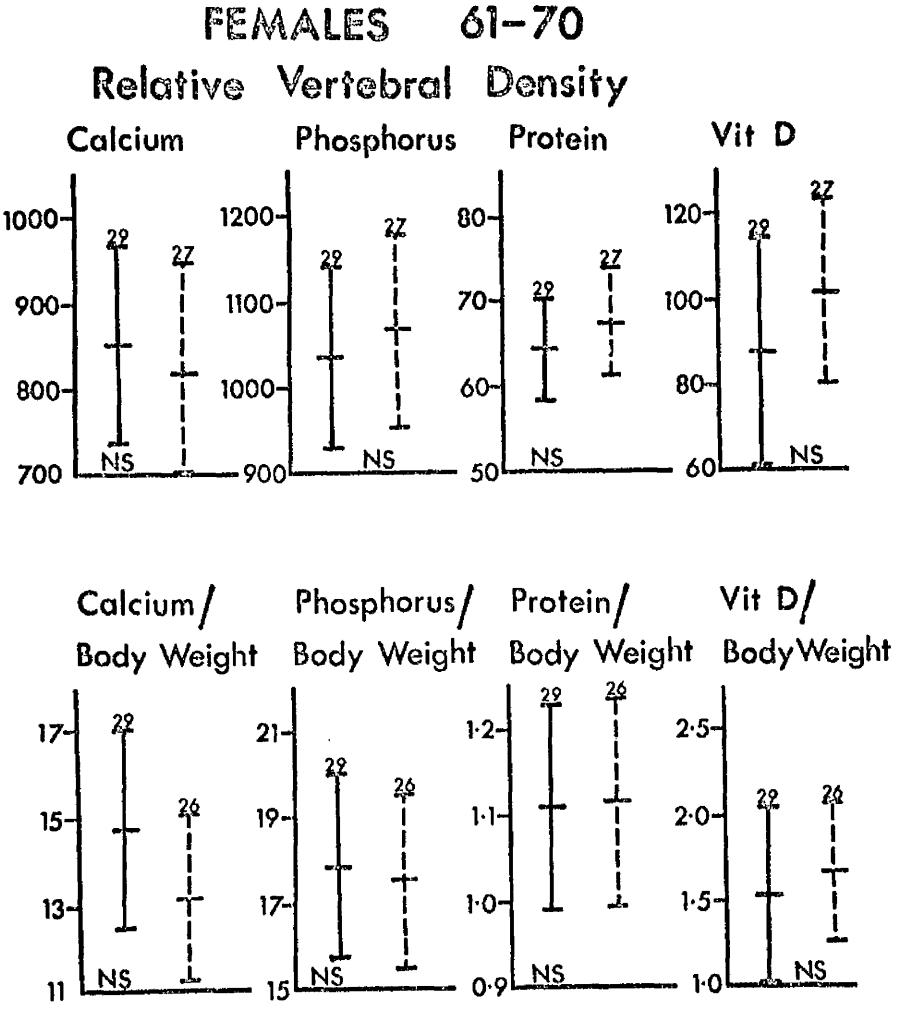


Fig 111 The dietary intake in women between 61 and 70 years of age with Relative Vertebral Densities above and below the mean value of the index. The mean and 2 S.E. range are shown.

The continuous line shows those below the mean R.V.D. and the interrupted line shows those above the mean R.V.D.

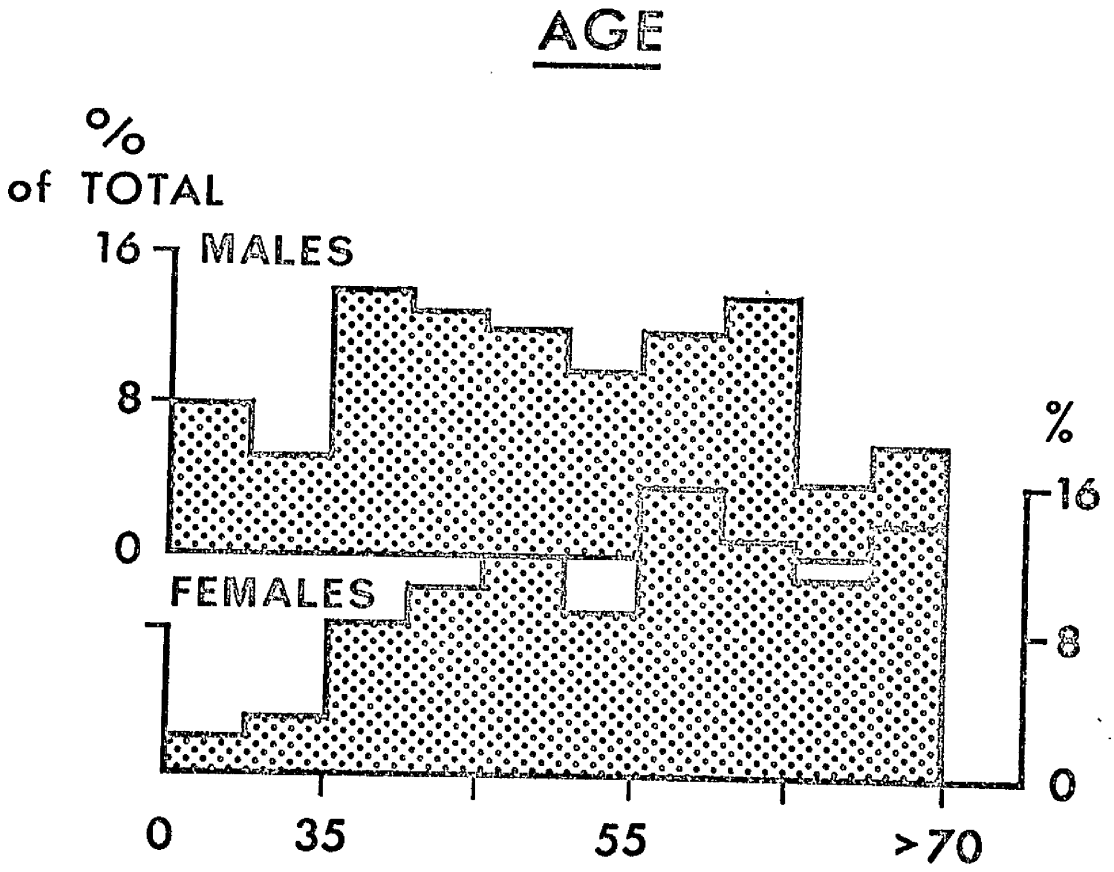


Fig 112 The age distribution of the normal male and female subjects expressed as a percentage of the total.

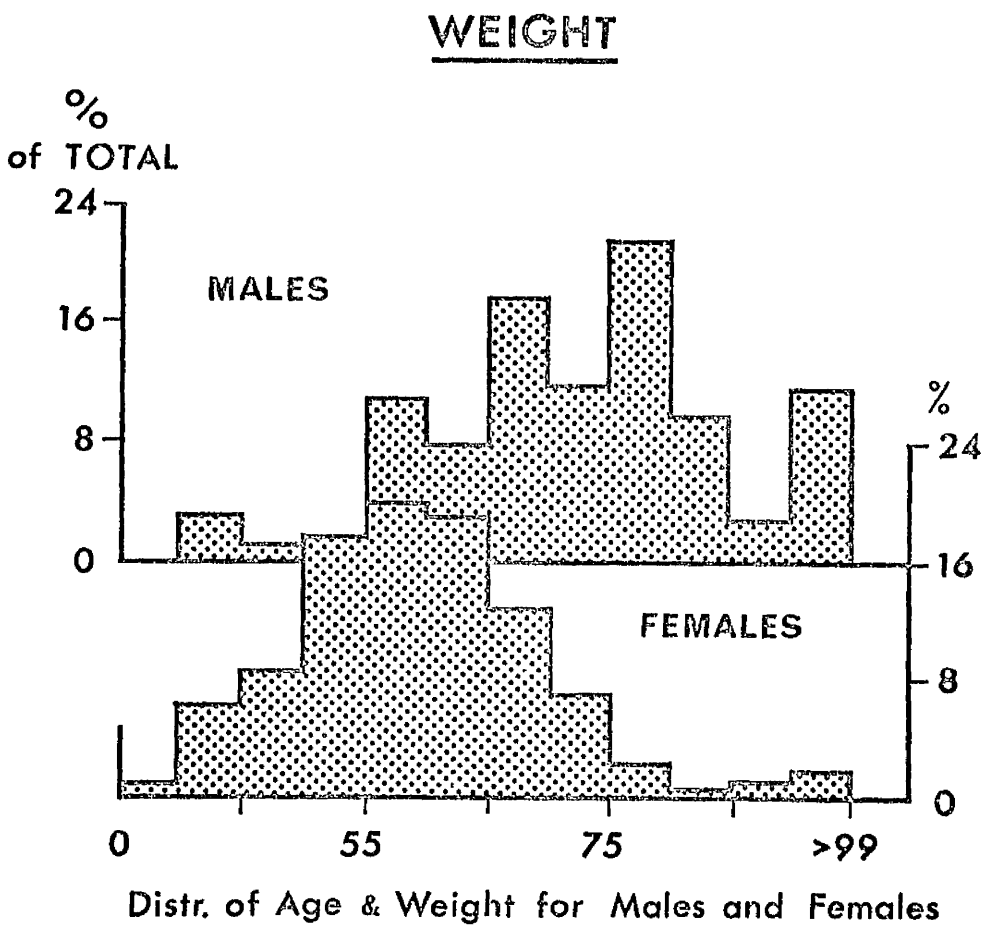


Fig 113 The weight distribution of the normal male and female subjects expressed as a percentage of the total.

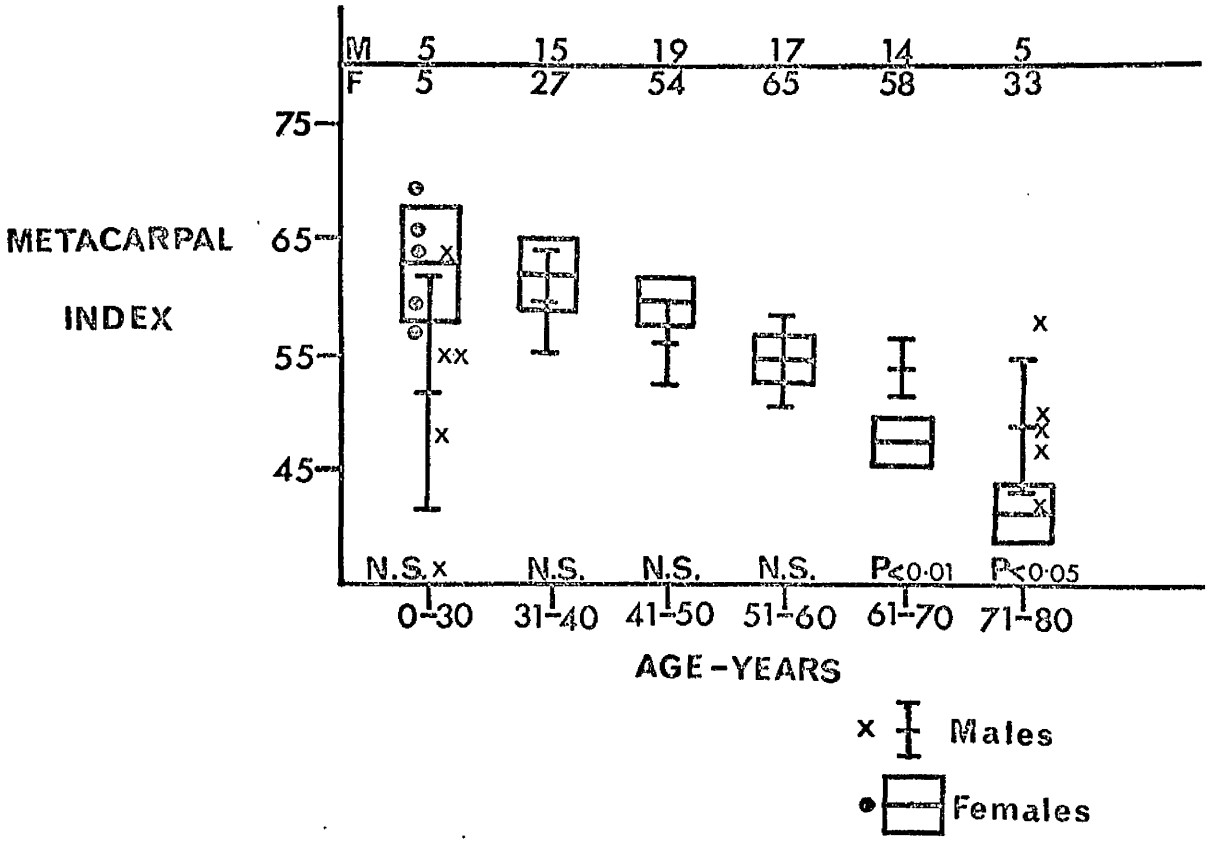


Fig 114 The comparison of the change in Metacarpal Index with age in men and women by decades. The mean and 2 S.E. range are shown.

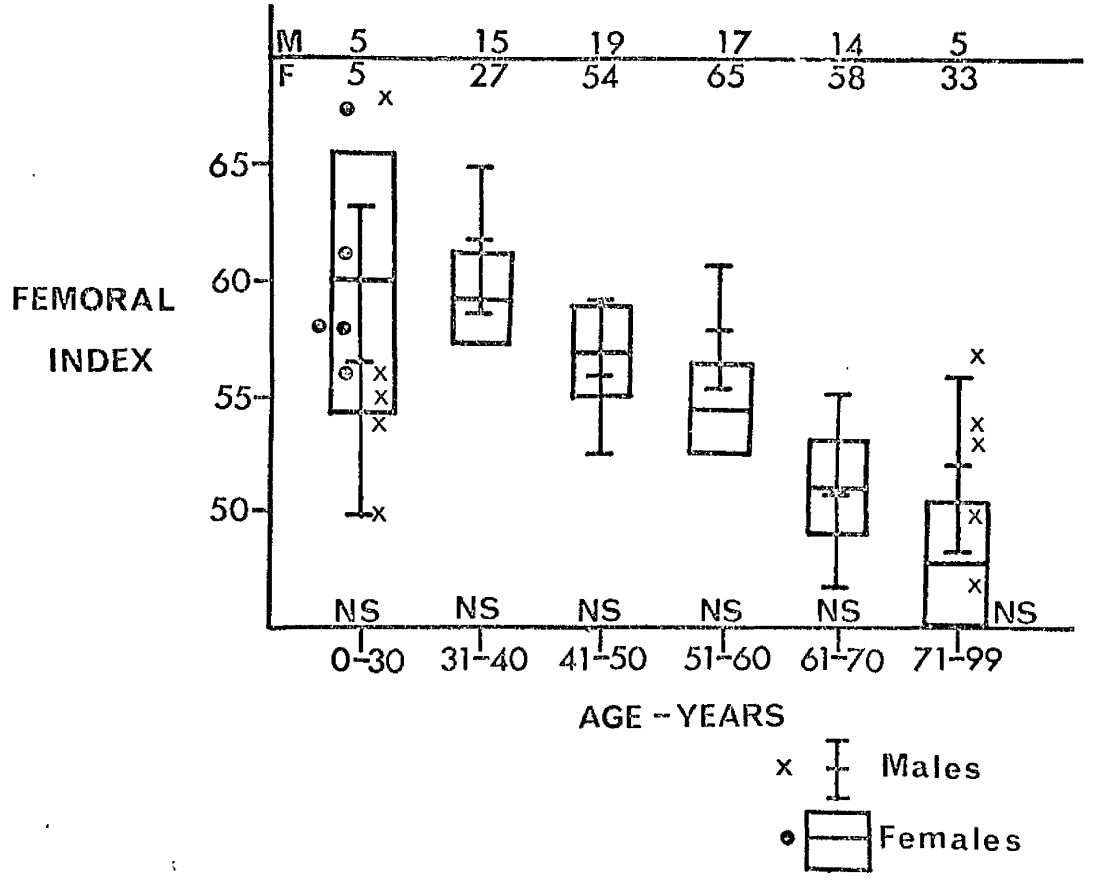


Fig 115 A comparison of the change in Femoral Index with age in men and women by decades. The mean and 2 S.E. range are shown.

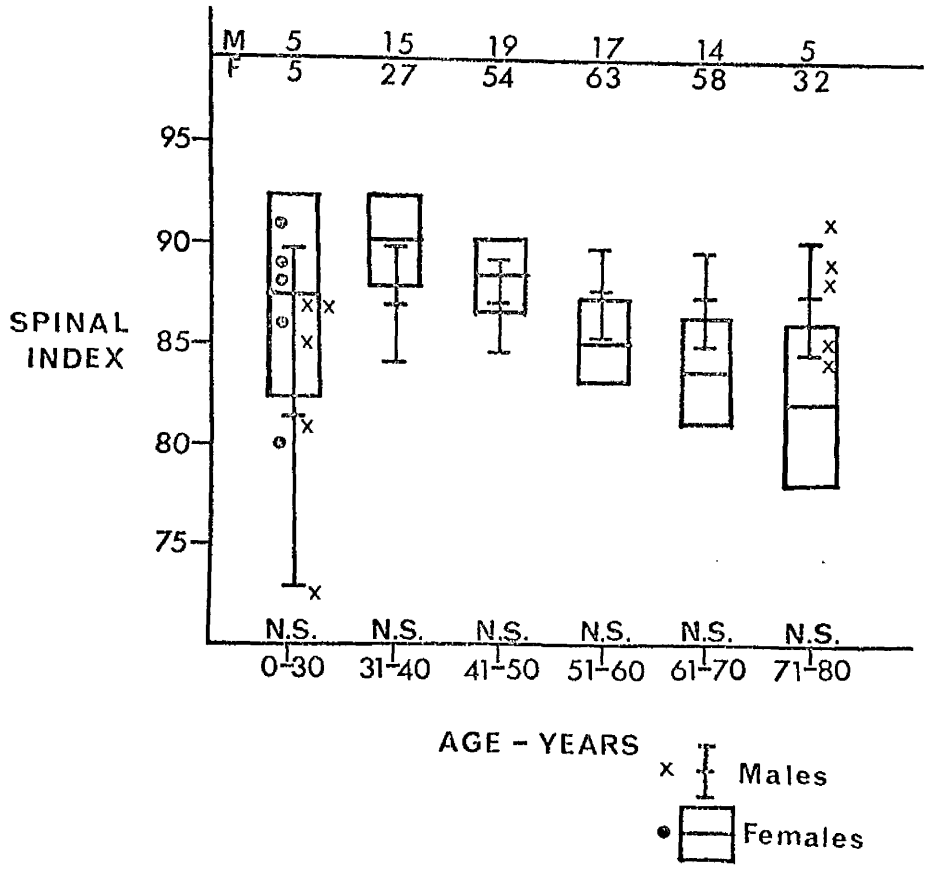


Fig 116 A comparison of the change in Spinal Index with age in men and women by decades. The mean and 2 S.E. range are shown.

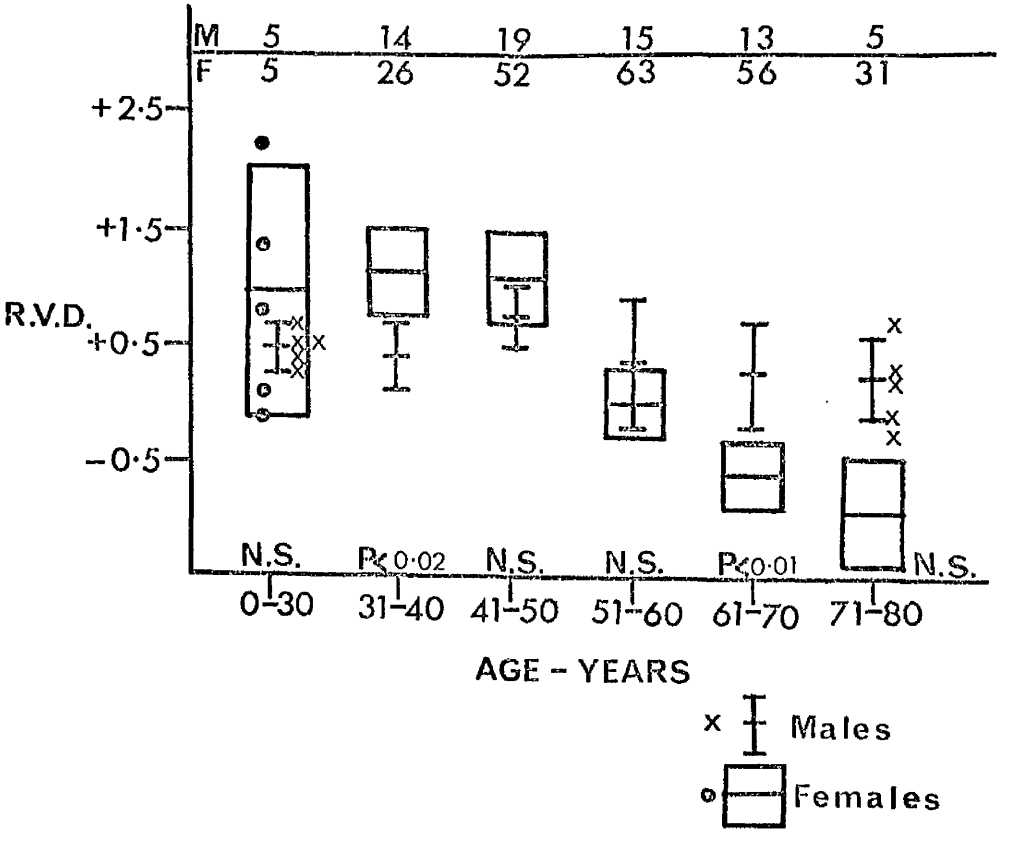


Fig 117 A comparison of the change in R.V.D. with age in men and women by decades. The mean and 2 S.E. range are shown.

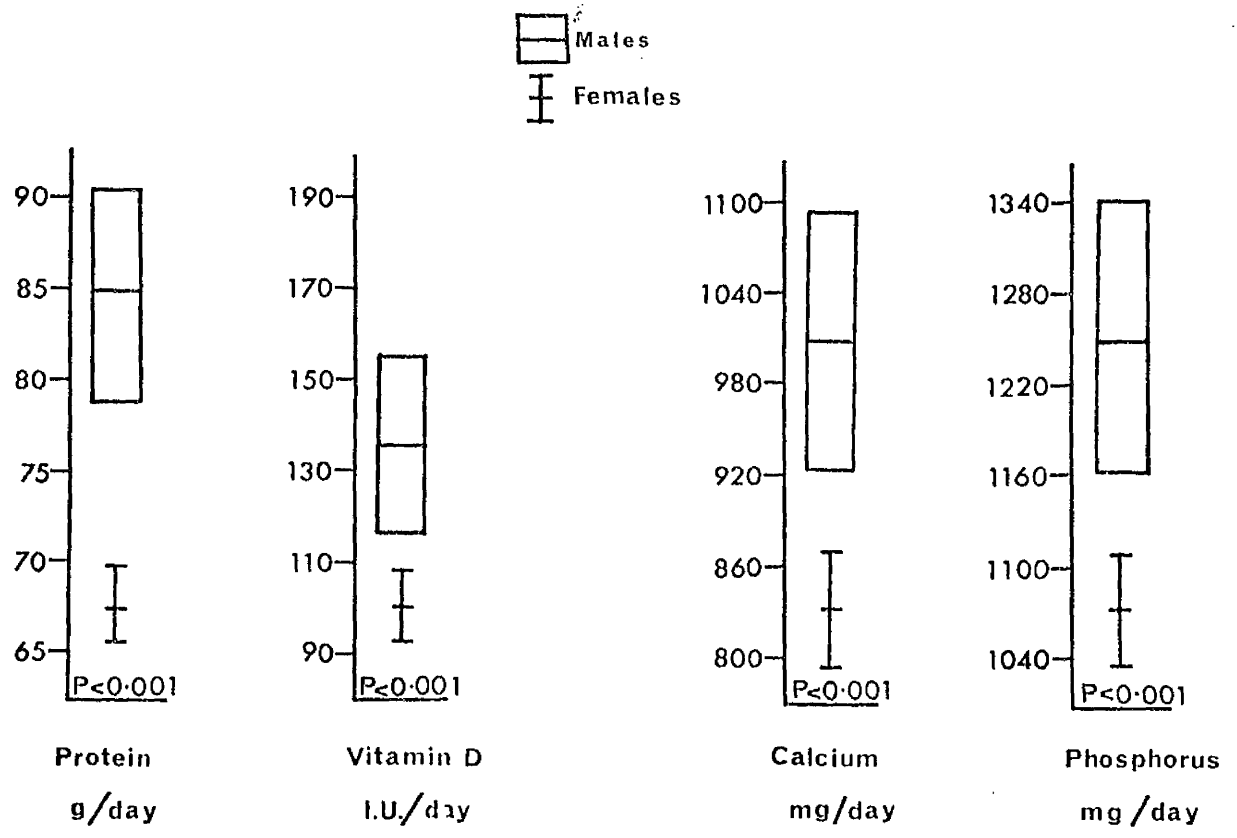


Fig 118 Dietary intake of calcium, phosphorus, protein and vitamin D in men and women. The mean and 2 S.E. range are shown.

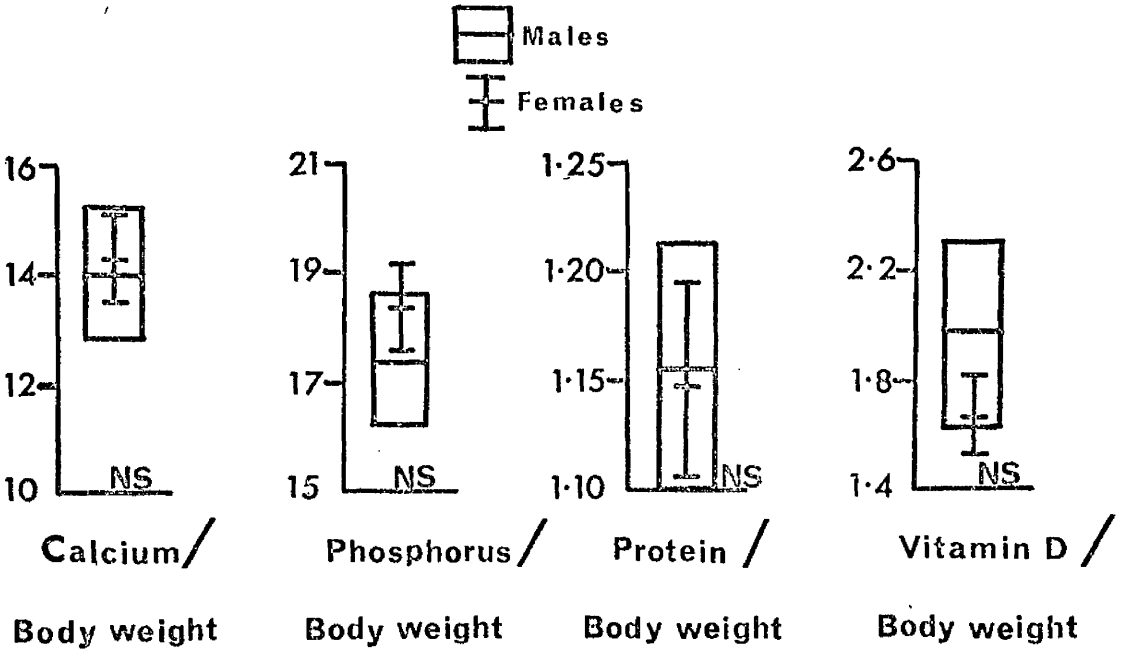


Fig 119 Dietary intake of calcium, phosphorus, protein and vitamin D divided by body weight in men and women. The mean and 2 S.E. range are shown.

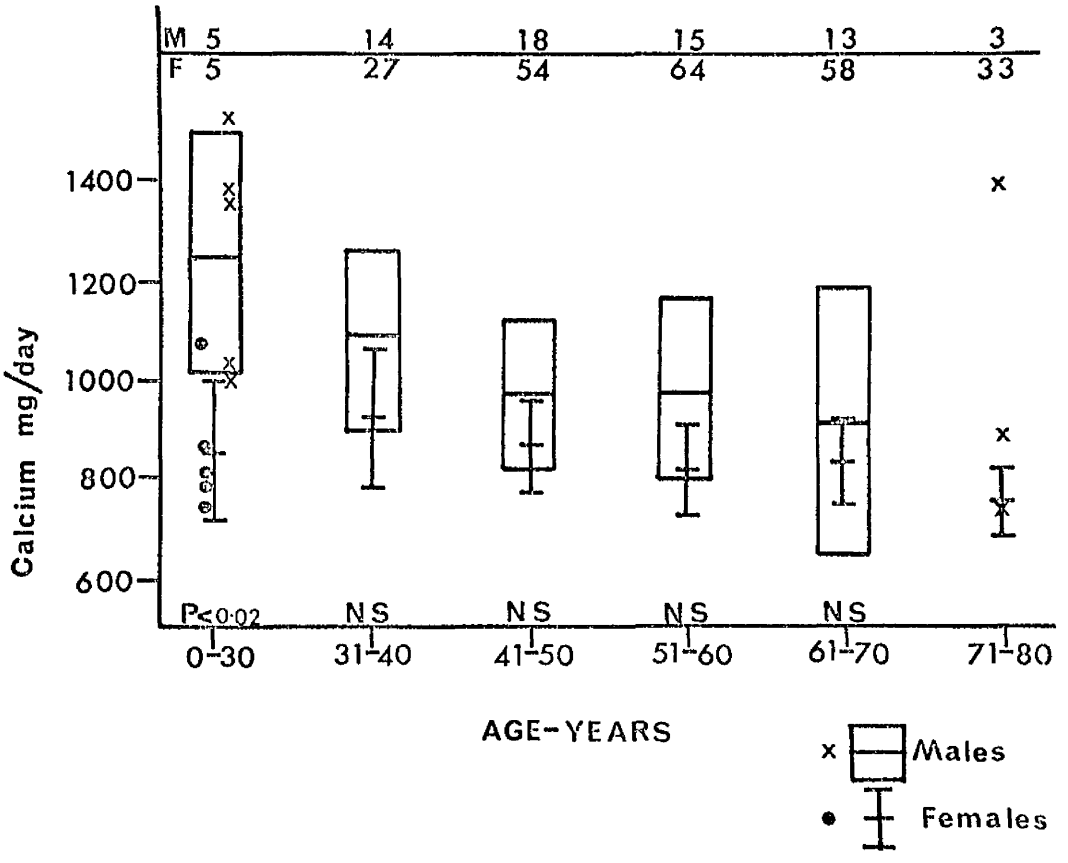


Fig 120 The dietary intake of calcium by decades in men and women. The mean and 2 S.E. range are shown.

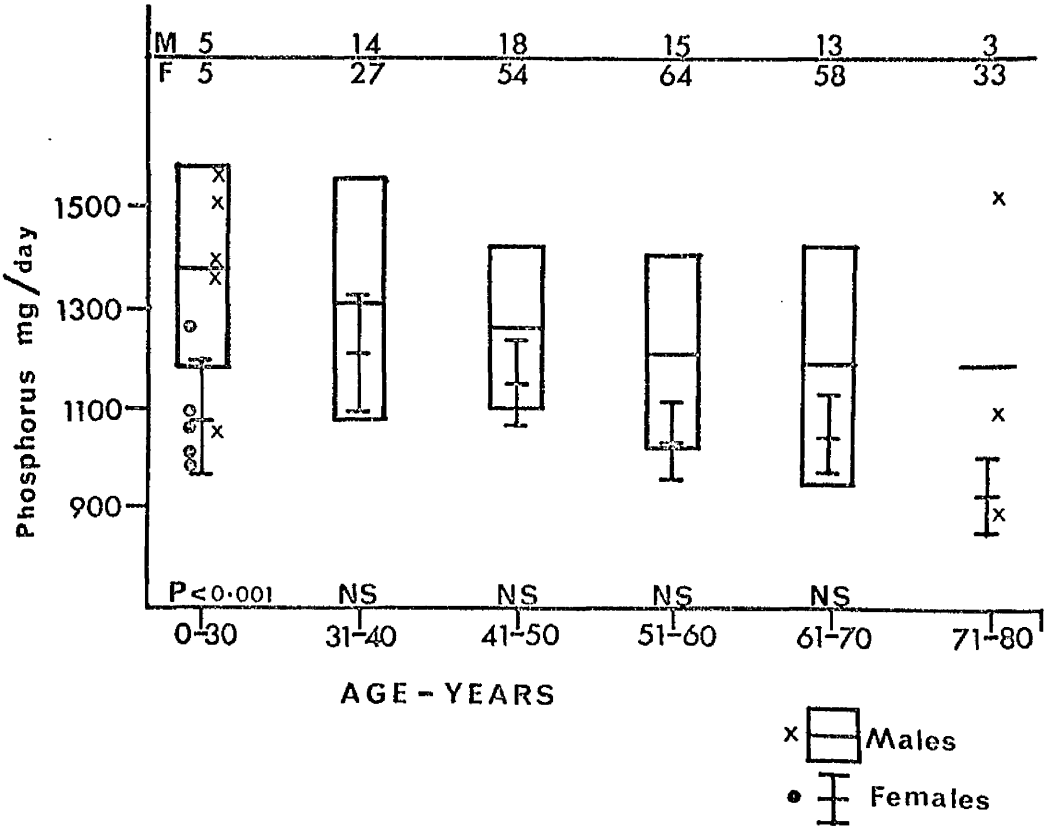


Fig 121 The dietary intake of phosphorus by decades in men and women. The mean and 2 S.E. range are shown.

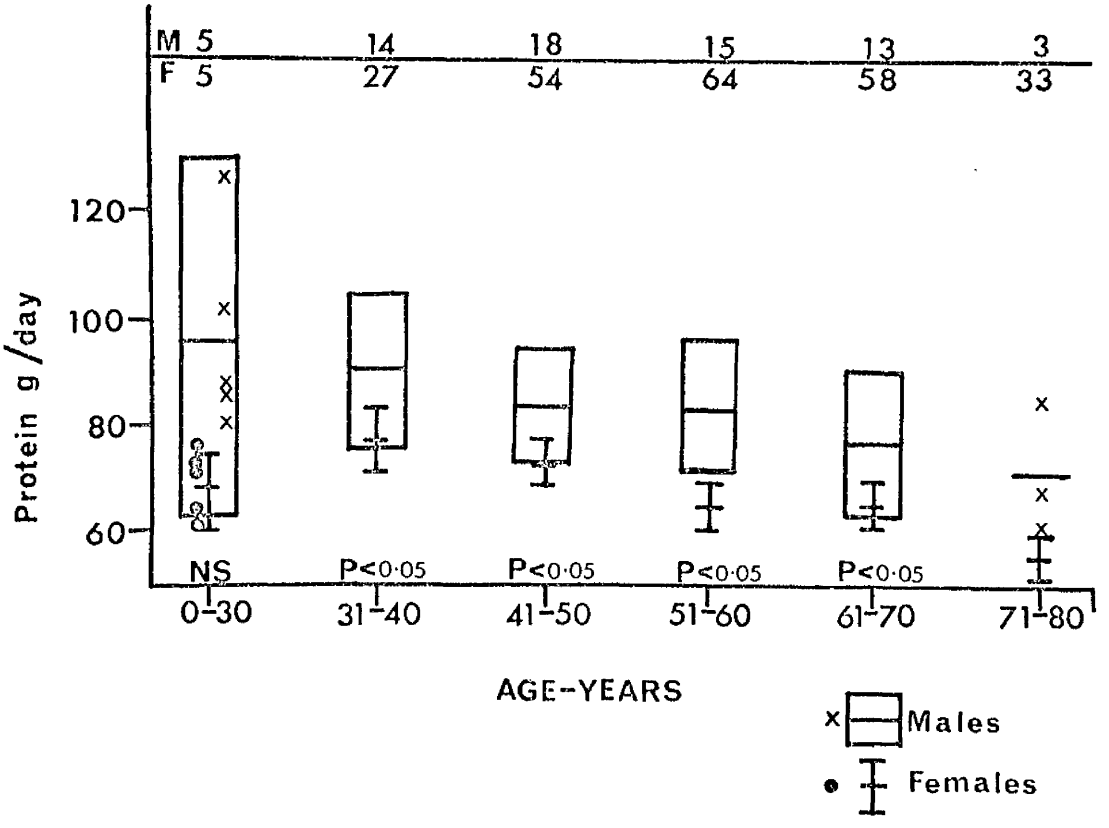


Fig 122 The dietary intake of protein by decades in men and women. The mean and 2 S.E. range are shown.

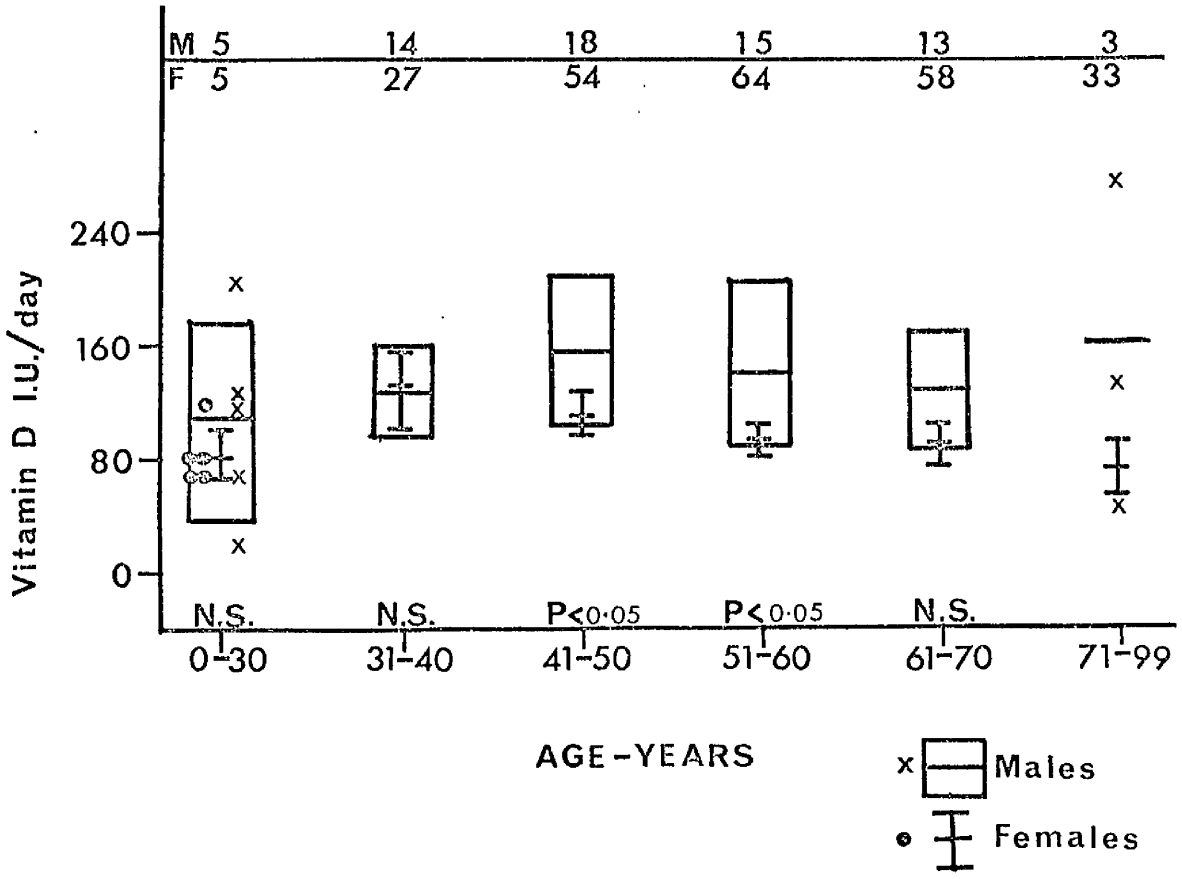


Fig 123 The dietary intake of vitamin D by decades in men and women. The mean and 2 S.E. range are shown.

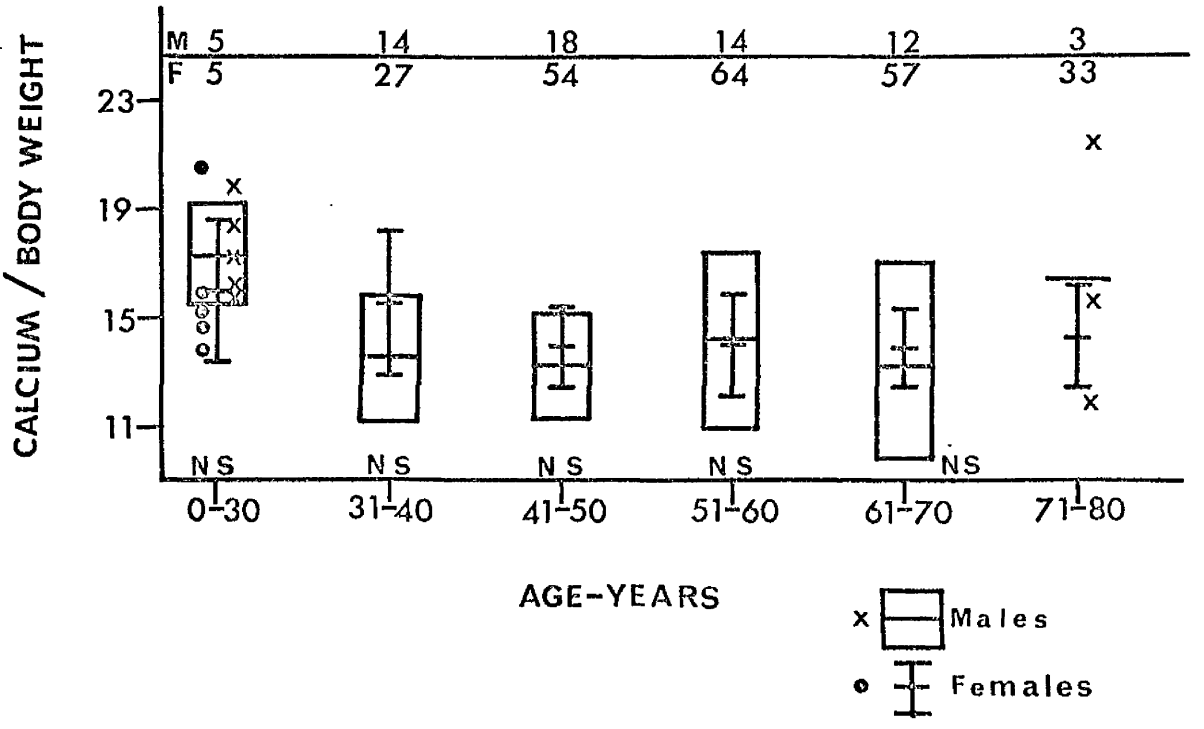


Fig 124 The dietary intake of calcium divided by body weight, by decades, in men and women. The mean and 2 S.E. range are shown.

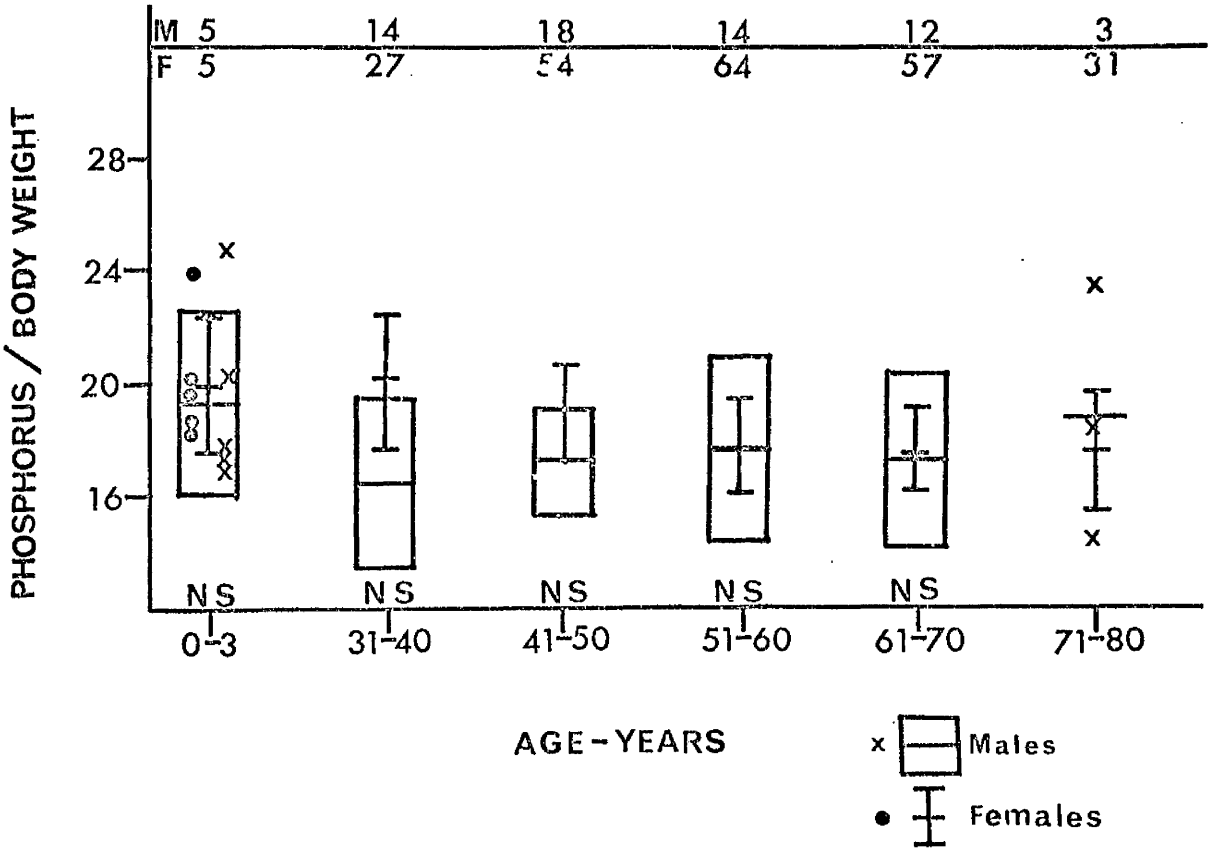


Fig 125 The dietary intake of phosphorus, divided by body weight, by decades, in men and women. The mean and 2 S.E. range are shown.

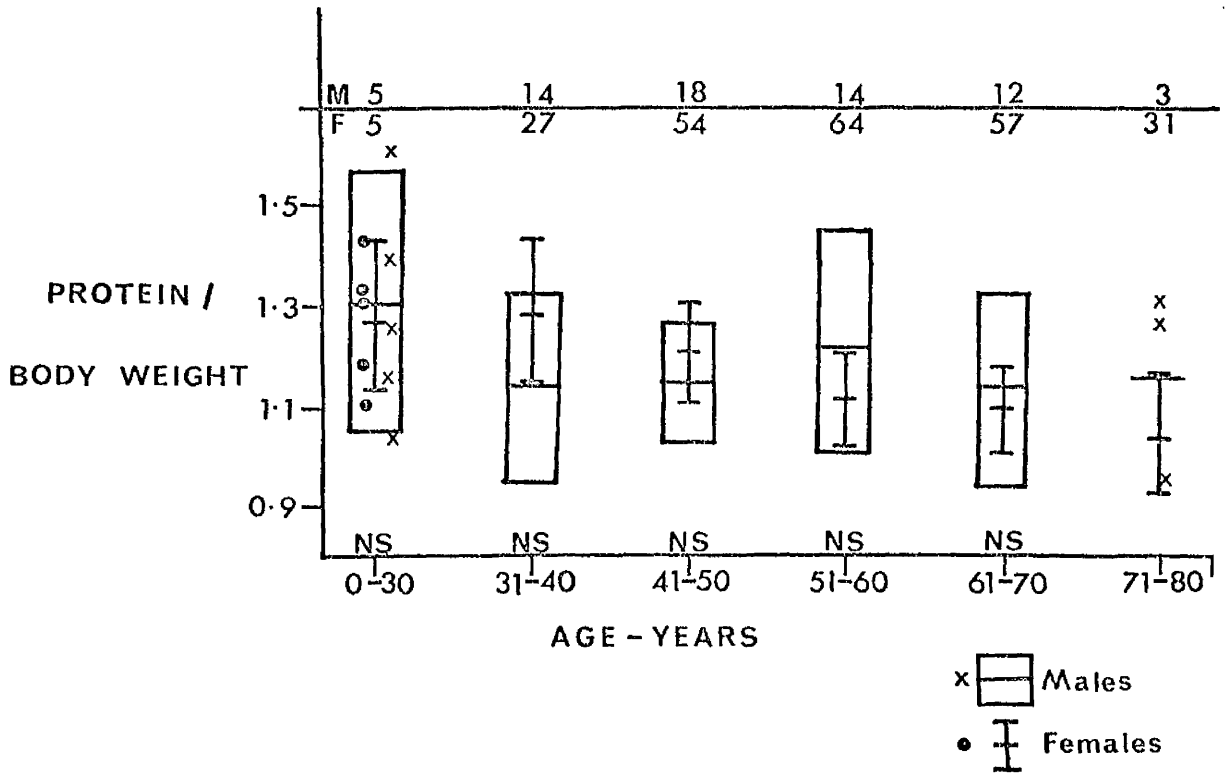


Fig 126 The dietary intake of protein, divided by body weight, by decades, in men and women. The mean and 2 S.E. range are shown.

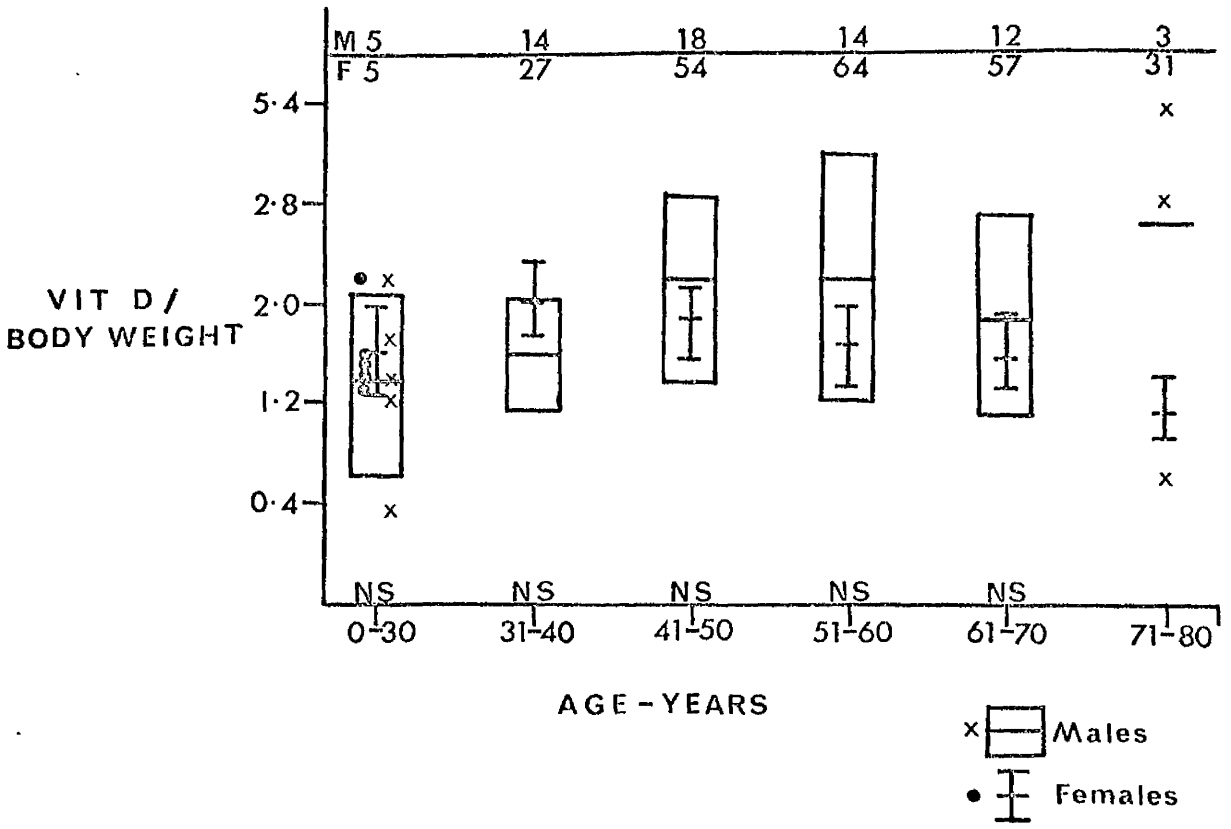


Fig 127 The dietary intake of vitamin D, divided by body weight, by decades, in men and women. The mean and 2 S.E. range are shown.

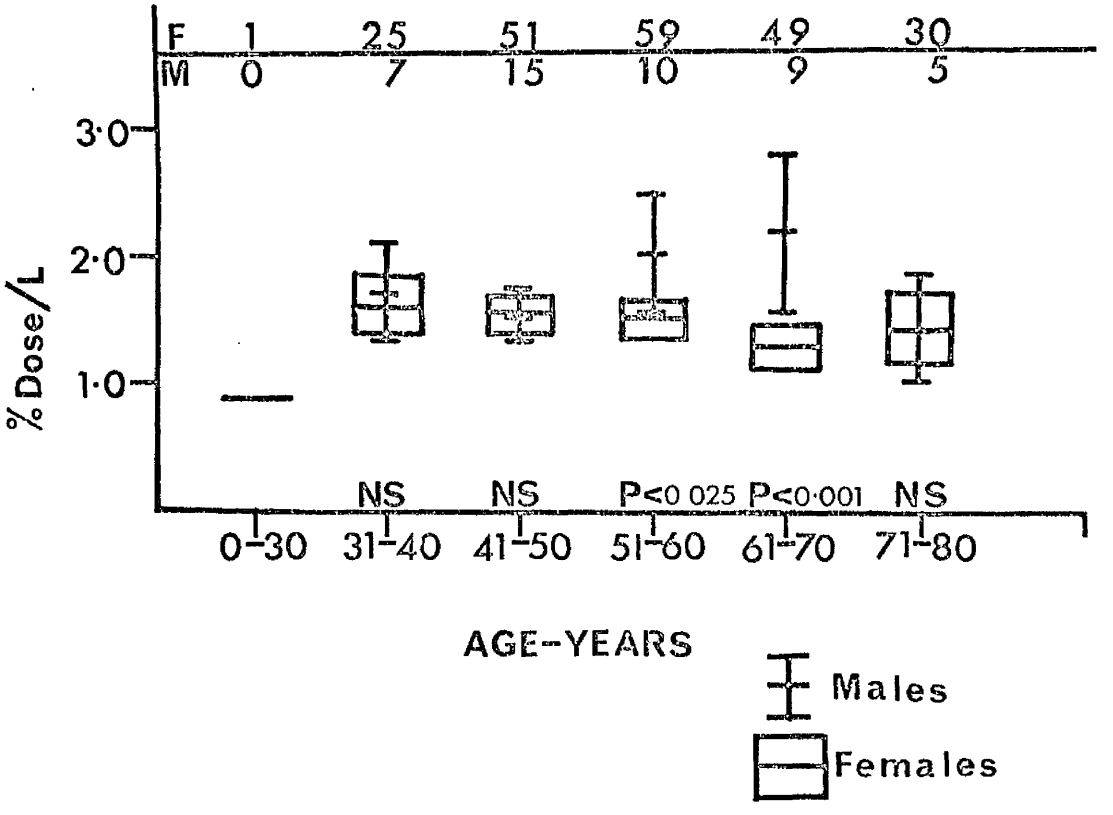


Fig 128 Calcium absorption measured as the specific activity two hours after the oral dose in men and women. The mean and 2 S.E. range by decades are shown.

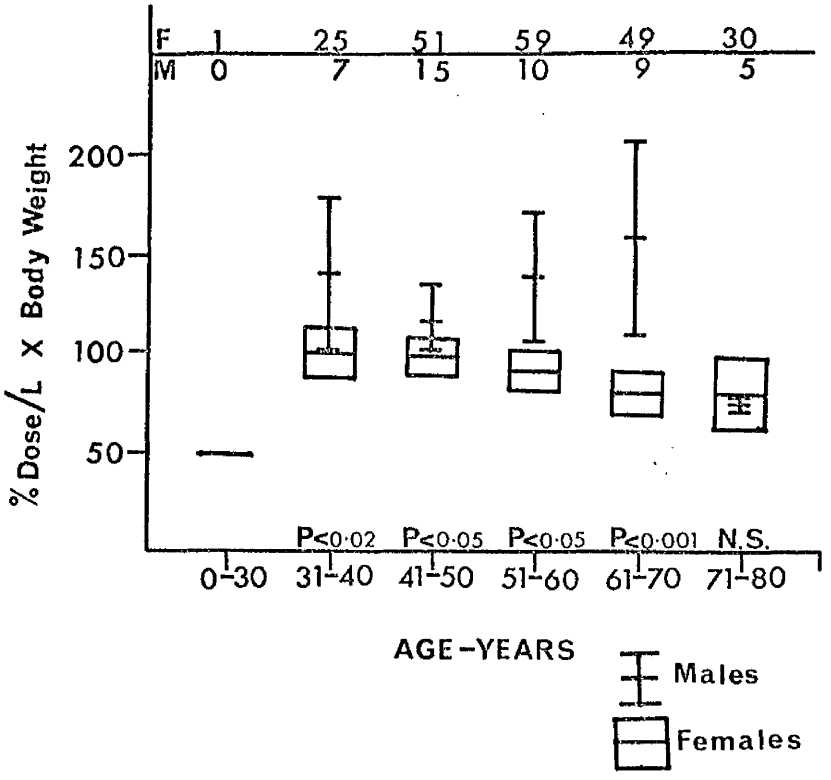


Fig 129 Calcium absorption measured as the specific activity adjusted for body weight, two hours after the oral dose, in men and women. The mean and 2 S.E. range are shown by decades.

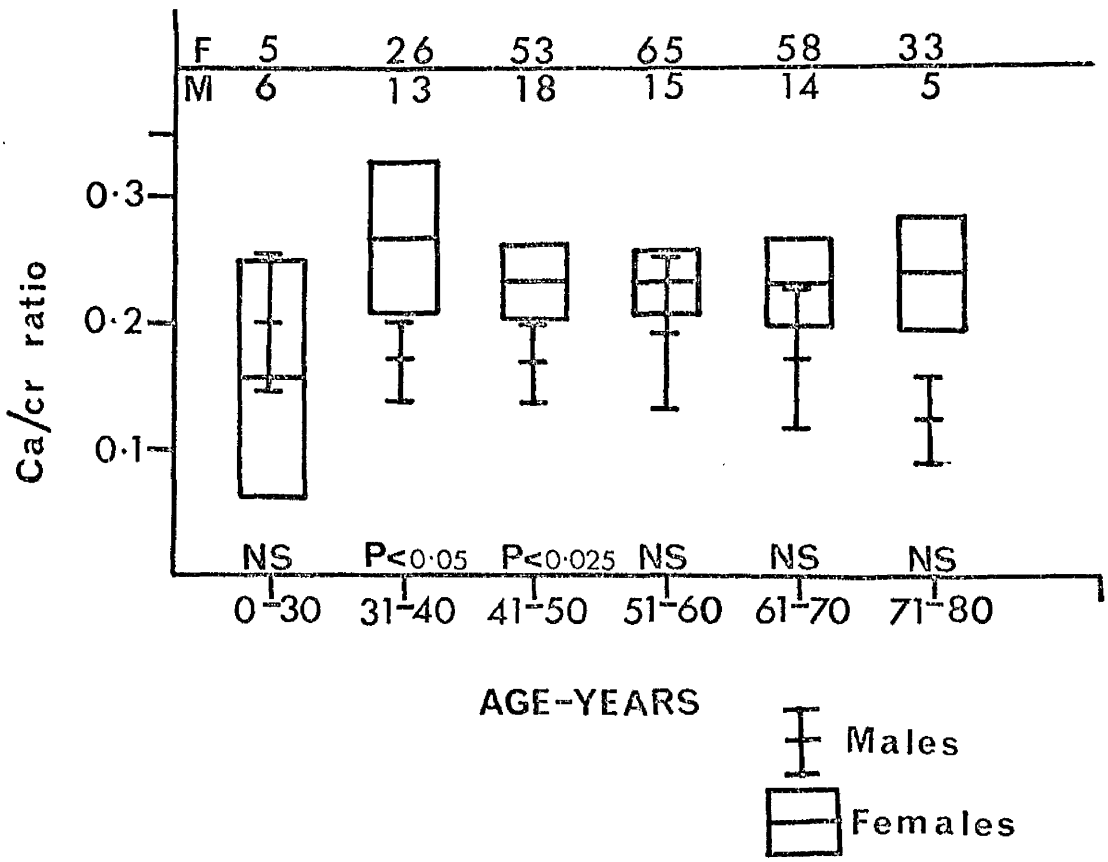


Fig 130 The urinary calcium excretion in men and women expressed as the mean of two estimations of the calcium/creatinine ratio. The mean and 2 S.E. range by decades are shown.

CHAPTER VIII

	Mean value ± Standard Deviation	
	Vagotomy with gastro-jejunostomy	Polyagastrectomy
Number of subjects	56	51
Height in cms	170.1 ± 6.9	169.0 ± 6.5
Weight in Kg	62.9 ± 7.2	61.1 ± 7.6
Months since operation	102.4 ± 17.1	104.7 ± 12.3

(None of the differences approached statistical significance).

TABLE XLVII

Age group	Dietary Constituent	Normal subjects v vagotomy + gastro-jejunostomy	Normal subjects v Polya-gastrectomy	Normal subjects v Vagotomy + gastro-jejunostomy & Polya-gastrectomy
31 - 40	Calcium (mg/day)	$P < 0.025$	-	-
41 - 50	Calcium (mg/day)	n.s.	n.s.	n.s.
51 - 60	Calcium (mg/day)	n.s.	n.s.	n.s.
61 - 70	Calcium (mg/day)	$P < 0.025$	< 0.01	n.s.
31 - 40	Calcium (mg/day/Kg body weight)	$P < 0.001$	n.s.	< 0.025
41 - 50	"	$P < 0.05$	< 0.005	< 0.001
51 - 60	"	n.s.	n.s.	n.s.
61 - 70	"	$P < 0.005$	n.s.	n.s.
31 - 40	Phosphorus (mg/day)	$P < 0.01$	n.s.	< 0.05
41 - 50	Phosphorus (mg/day)	n.s.	< 0.05	< 0.05
51 - 60	Phosphorus (mg/day)	n.s.	n.s.	n.s.
61 - 70	Phosphorus (mg/day)	$P < 0.05$	n.s.	n.s.
31 - 40	Phosphorus (mg/day/Kg body weight)	$P < 0.001$	n.s.	< 0.005
41 - 50	"	$P < 0.02$	< 0.001	< 0.001
51 - 60	"	$P < 0.05$	n.s.	< 0.02
61 - 70	"	$P < 0.005$	< 0.01	< 0.01

TABLE XLVIII

Comparison of dietary intakes of calcium and phosphorus between normal subjects and patients who had undergone vagotomy with gastro-jejunostomy and Polya-gastrectomy.

n.s. = not significant.

	Patient	Age	Ca	P	Alk. Phos.	Ca/Cr	P.E.I.	M.I.	Repeat alkaline phosphatase	
1) Patients with vagotomy and gastro-jejunostomy with raised alkaline phosphatase	J.N.	50	10.1	3.7	14	0.15	-0.06	52	6	8
	H.O.	51	10.6	3.9	21	0.05	-0.07	58		D.N.A.
	A.K.	42	9.9	3.1	14	0.12	+0.08	47	10	9
	R.M.	56	10.4	3.5	26	0.17	+0.08	42	19	30
	S.K.	45	9.0	3.5	13	0.13	+0.06	57	12	11
2) Patients with polya-gastrectomy with raised alkaline phosphatase	R.A.	64	9.8	3.1	13	0.06	+0.09	40	8	8
	T.B.	53	8.8	2.6	13	0.11	+0.07	43	10	11
	R.W.	47	9.8	3.0	14	0.07	+0.03	57	9	8
	W.S.	64	8.4	2.6	17	0.12	+0.11	44	16	14
	J.D.	41	10.3	3.7	13	0.25	-0.09	46	12	10
3) Patient with vagotomy and gastro-jejunostomy with low plasma calcium	W.B.	53	8.8	3.5	6	0.09	-0.06	-		d.n.a.
4) Patients with polya-gastrectomy with low plasma calcium	T.H.	60	8.6	3.4	12	0.09	+0.09	36		d.n.a.
	T.B.	53	8.8	2.6	13	0.11	+0.07	43	9.6	10.0
	W.S.	47	8.4	2.6	17	0.12	+0.11	44	8.7	8.5
	T.C.	41	8.7	3.6	8	0.07	+0.09	66	8.9	9.0
	R.E.	51	8.7	2.9	7	0.11	+0.05	-		d.n.a.

TABLE XLVIX

Biochemical results in patients who have had gastric surgery and who have one or more abnormal values.

	Patient	Age	Ca	P	Alk. Phos.	Ca/Cr	P.E.I.	M.I.	Total 'P' intake	P intake Body Wt.	Repeat	P.E.I.
Patients with vagotomy and jejunostomy with raised P.E.I.	C.N.	63	10.1	2.4	11	0.10	+0.15	56	1279	20.9	+ 0.14	+ 0.15
	J.F.	56	10.0	3.3	8	0.20	+0.25	–	860	11.8	+ 0.01	– 0.01
	A.W.	52	10.0	2.5	9	0.09	+0.21	–	–	–	+ 0.05	– 0.02
	A.S.	42	9.2	3.8	5	0.30	+0.37	52	1466	22.8	+ 0.10	+ 0.03
	J.K.	33	9.8	3.1	5	0.15	+0.17	–	1723	25.4	– 0.01	–
	J.F.	36	9.5	3.0	12	0.19	+0.26	52	1698	25.5	+ 0.06	– 0.04
	T.T.	58	9.7	3.0	8	0.10	+0.17	54	2454	31.3	+ 0.09	–
	R.C.	45	10.0	2.7	8	0.09	+0.19	62	1663	20.4	– 0.06	+ 0.04
	D.M.	57	9.8	3.8	7	0.26	+0.12	–	1662	19.5	+ 0.02	–
	A.C.	35	9.8	3.1	12	0.26	+0.10	–	2071	28.8	+ 0.02	–
	J.M.	67	10.1	2.6	10	0.19	+0.15	39	1618	28.1	–0.03	+ 0.09
Patients with polya-gastrectomy with raised P.E.I.	J.M.	51	10.6	4.1	7	0.14	+0.12	57	966	20.3	D.N.A.	
	A.T.	54	9.9	3.4	6	0.06	+0.37	52	1204	16.7	– 0.03	– 0.10
	A.Y.	51	9.3	3.7	8	0.18	+0.21	–	–	–	– 0.06	–
	D.M.	53	9.8	3.1	9	0.18	+0.15	–	888	14.5	+ 0.07	+ 0.09
	A.S.	62	9.5	3.0	10	0.19	+0.14	46	1692	24.7	+ 0.08	+ 0.04
	F.G.	47	9.7	3.9	9	0.33	+0.13	55	2091	30.5	– 0.08	–
	R.T.	47	10.1	4.3	11	0.29	+0.11	46	1438	24.2	–0.01	– 0.04
	L.B.	53	9.8	3.1	11	0.06	+0.15	50	1584	26.1	+0.17	+ 0.26
	J.H.	48	9.5	3.2	10	0.37	+0.15	52	1020	17.4	+0.06	+ 0.07
	I.S.	46	9.7	3.6	9	0.23	+0.10	48	1995	36.3	D.N.A.	
	D.M.	61	9.6	3.5	12	0.05	+0.13	33	1440	19.3	+0.04	–
	W.M.	57	9.7	3.3	11	0.23	+0.15	49	1385	14.9	+0.07	–
	W.S.	64	8.6	2.6	17	0.12	+0.11	44	1158	21.4	+0.16	+ 0.10
	A.S.	55	10.0	4.2	10	0.29	+0.18	62	1367	26.2	D.N.A.	
	J.T.	64	8.9	2.7	9	0.24	+0.11	52	966	20.3	D.N.A.	

TABLE L

Biochemical results in patients who have had gastric surgery and who have one or more abnormal values

X-Ray Indices		Diagnoses	n	r	Significance	Gradient
Metacarpal Index	(1)	Normal	75	-0.190	n.s.	-
	(2)	Vagotomy and gastro-jejunostomy	36	-0.401	P < 0.01	-0.430
	(3)	Polya-gastrectomy	43	-0.411	P < 0.01	-0.395
	(4)	Combined results for groups (2) and (3)	79	-0.408	P < 0.01	-0.413
Femoral Index	(1)	Normal	75	-0.320	P < 0.01	-0.174
	(2)	Vagotomy and gastro-jejunostomy	34	-0.057	n.s.	-
	(3)	Polya-gastrectomy	37	-0.20	n.s.	-
	(4)	Combined results for groups (2) and (3)	71	-0.135	n.s.	-
Spinal Index	(1)	Normal	75	-0.130	n.s.	-
	(2)	Vagotomy and gastro-jejunostomy	33	-0.087	n.s.	-
	(3)	Polya-gastrectomy	36	-0.103	n.s.	-
	(4)	Combined results for groups (2) and (3)	69	-0.046	n.s.	-

TABLE LI

The relation between X-ray indices of bone mass and age in normal male subjects and in male patients who had undergone gastric surgery for peptic ulcer.

Diagnostic groups	Age range	P Metacarpal Index		P Femoral Index		P Spinal Index	
Vagotomy with gastro-jejunostomy v Polya-gastrectomy	31 - 40	-	*	-	*	-	*
	41 - 50	n.s.		n.s.		n.s.	
	51 - 60	n.s.		n.s.		n.s.	
	61 - 70	-	*	-	*	-	*
Vagotomy with gastro-jejunostomy v Normal subjects	31 - 40	-	*	-	*	-	*
	41 - 50	n.s.		n.s.		n.s.	
	51 - 60	< 0.05		< 0.01		n.s.	
	61 - 70	-	*	-	*	n.s.	
Polya-gastrectomy v Normal subjects	31 - 40	-	*	-	*	-	*
	41 - 50	< 0.05		n.s.		0.05	
	51 - 60	n.s.		n.s.		n.s.	
	61 - 70	P < 0.05		-	*	-	*
Polya-gastrectomy plus vagotomy with gastro-jejunostomy v Normal subjects	31 - 40	n.s.		-	**	-	**
	41 - 50	n.s.		n.s.		n.s.	
	51 - 60	< 0.01		< 0.01		n.s.	
	61 - 70	< 0.05		n.s.		n.s.	

* Numbers too small
** Variances significantly different

TABLE LII

The difference between the X-ray indices in the patients who had undergone vagotomy and gastro-jejunostomy. The difference between these patient groups and the normal subjects, the patient groups each being taken separately and together.

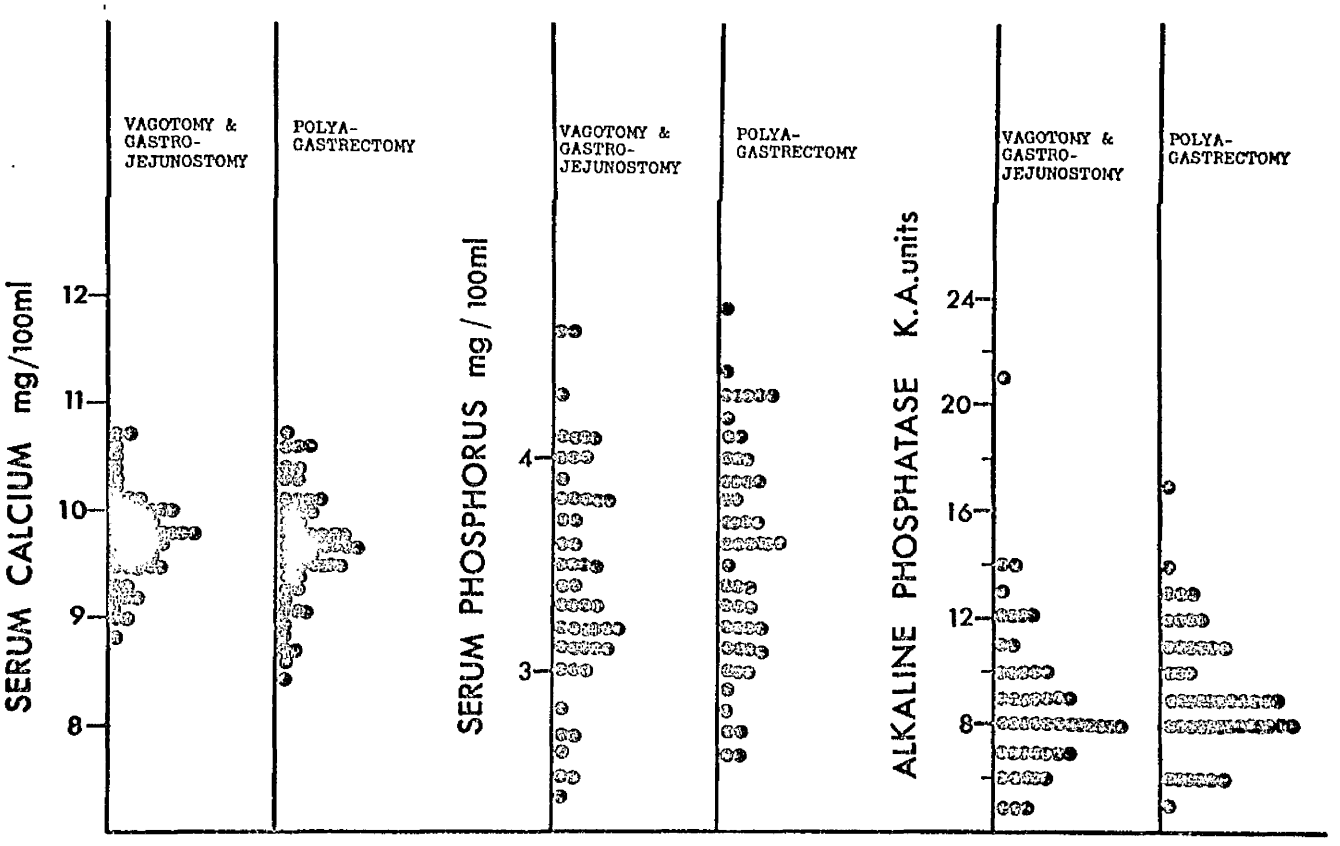


Fig 131 The serum calcium, phosphorus and alkaline phosphatase in patients who had undergone vagotomy with gastro-jejunostomy or Polya-gastrectomy 8 to 12 years prior to the estimation. The mean and 2 S.E. range by decades are shown.

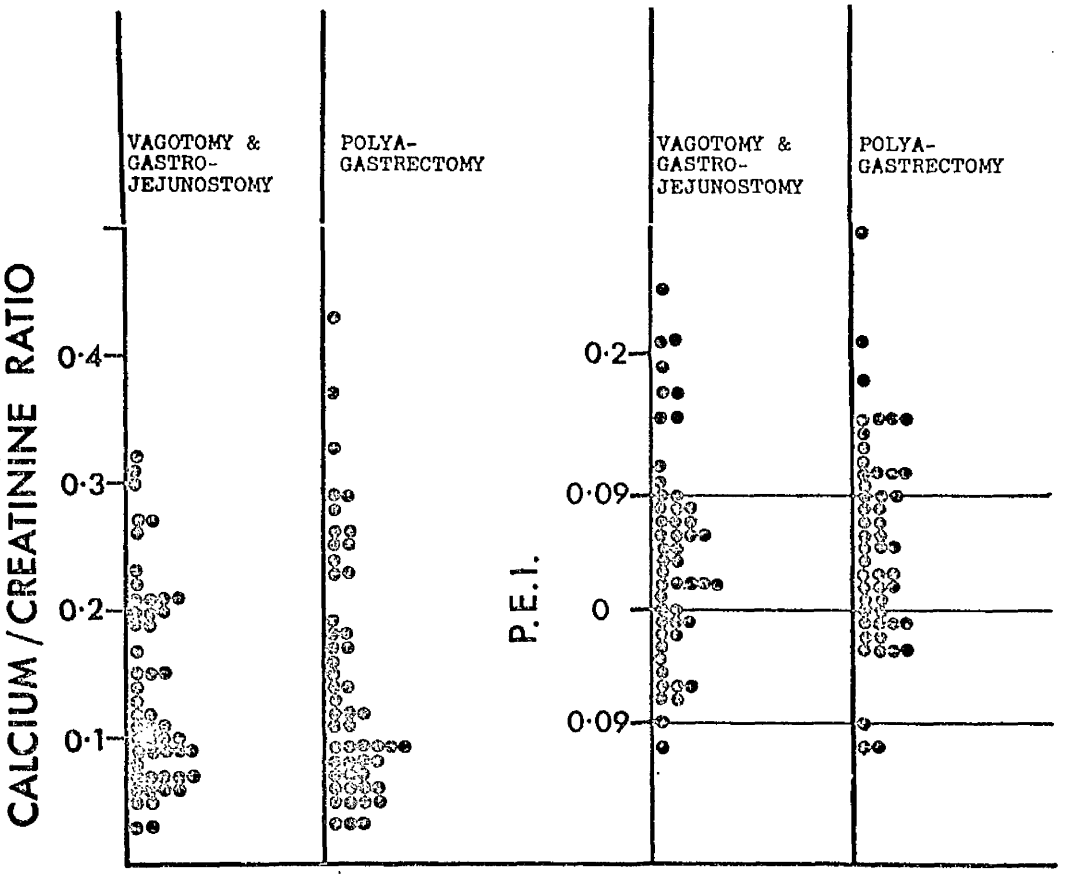


Fig 132 The calcium/creatinine ratio and the P.E.I. (Nordin and Fraser, 1960) in patients who had undergone vagotomy and gastro-jejunostomy or Polya-gastrectomy. The mean and 2 S.E. range by decades are shown.

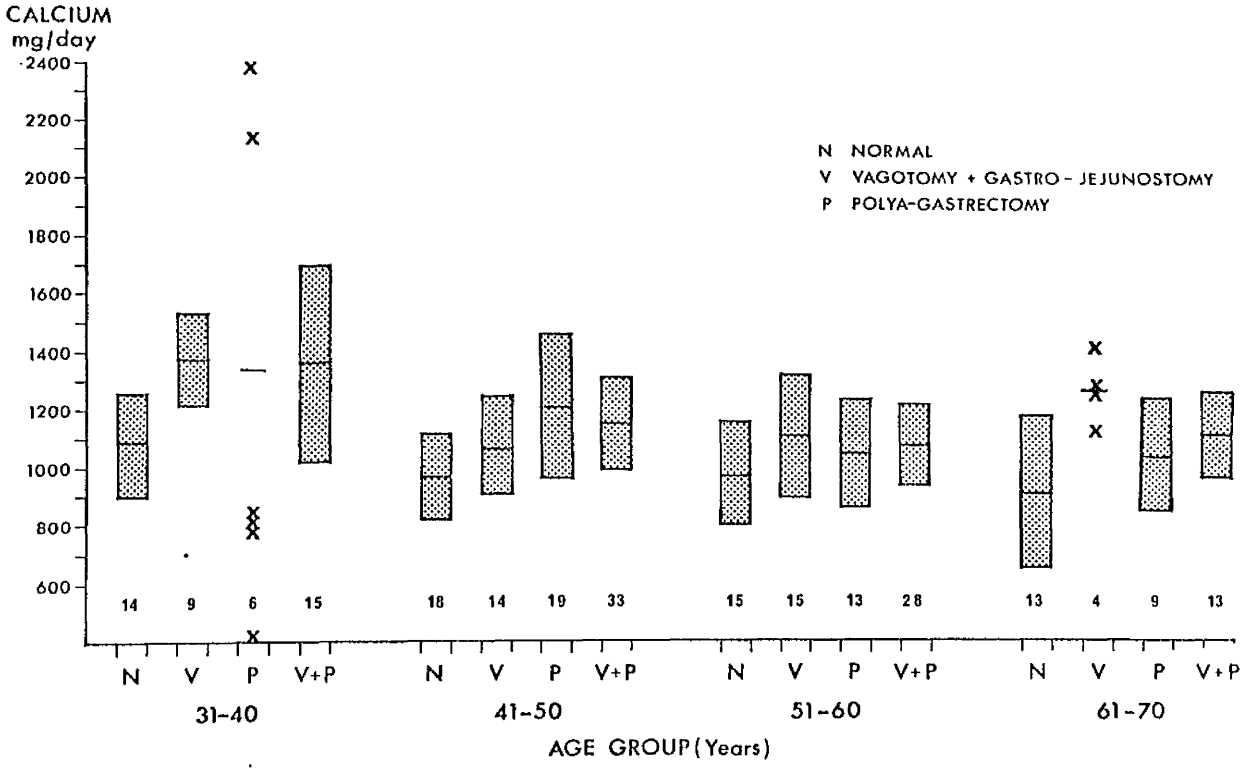


Fig 133 The dietary intake of calcium by decades in patients who have undergone vagotomy and gastro-jejunostomy or Polya-gastrectomy, compared with normal male subjects. The mean and 2 S.E. range are shown.

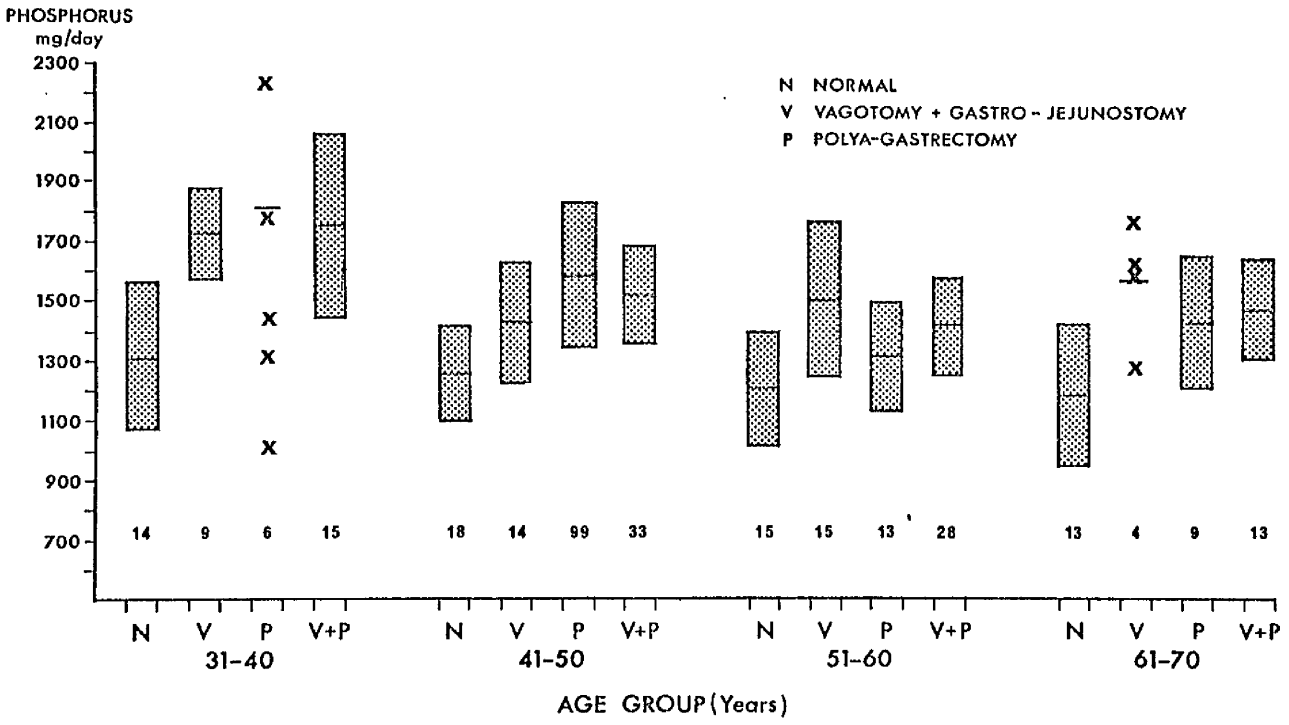


Fig 134 The dietary intake of phosphorus by decades in patients who have undergone vagotomy and gastro-jejunostomy or Polya-gastrectomy, compared with normal male subjects. The mean and 2 S.E. range are shown.

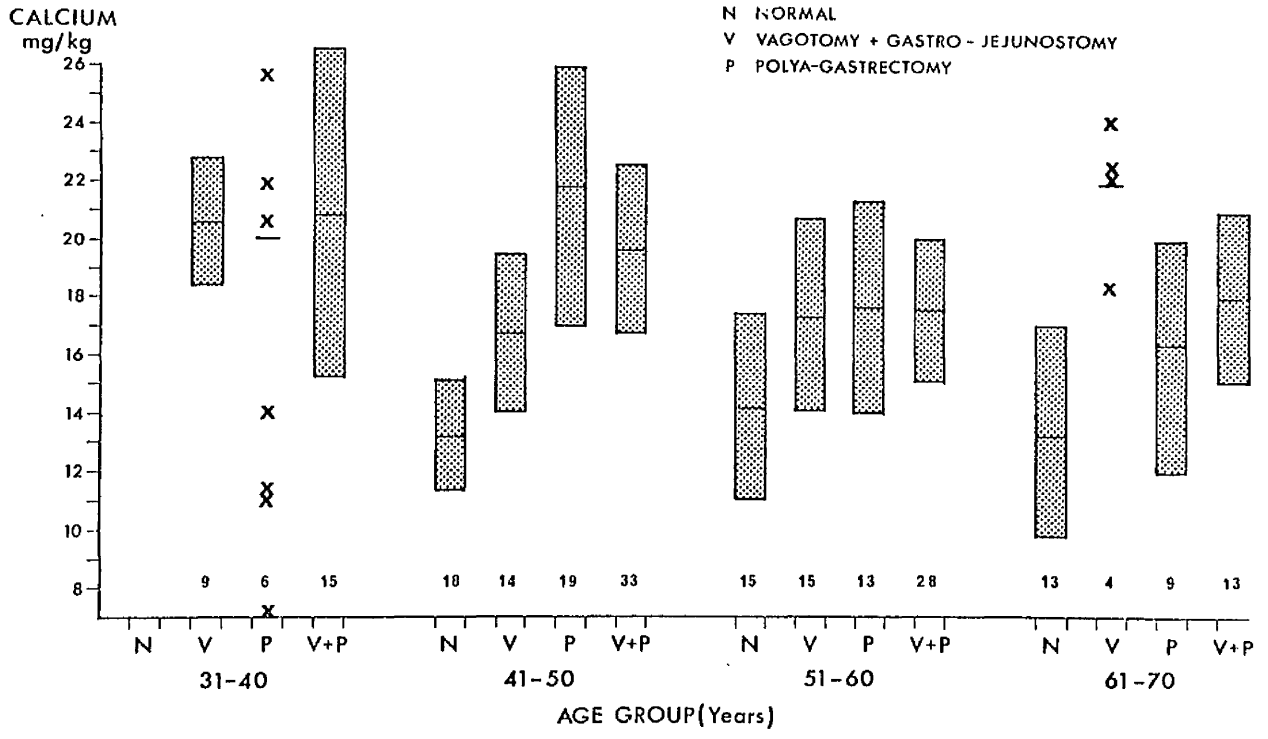


Fig 135 The dietary intake of calcium, divided by body weight, in patients who have undergone vagotomy with gastro-jejunostomy or Polya-gastrectomy, compared with normal subjects. The mean and 2 S.E. range by decades are shown.

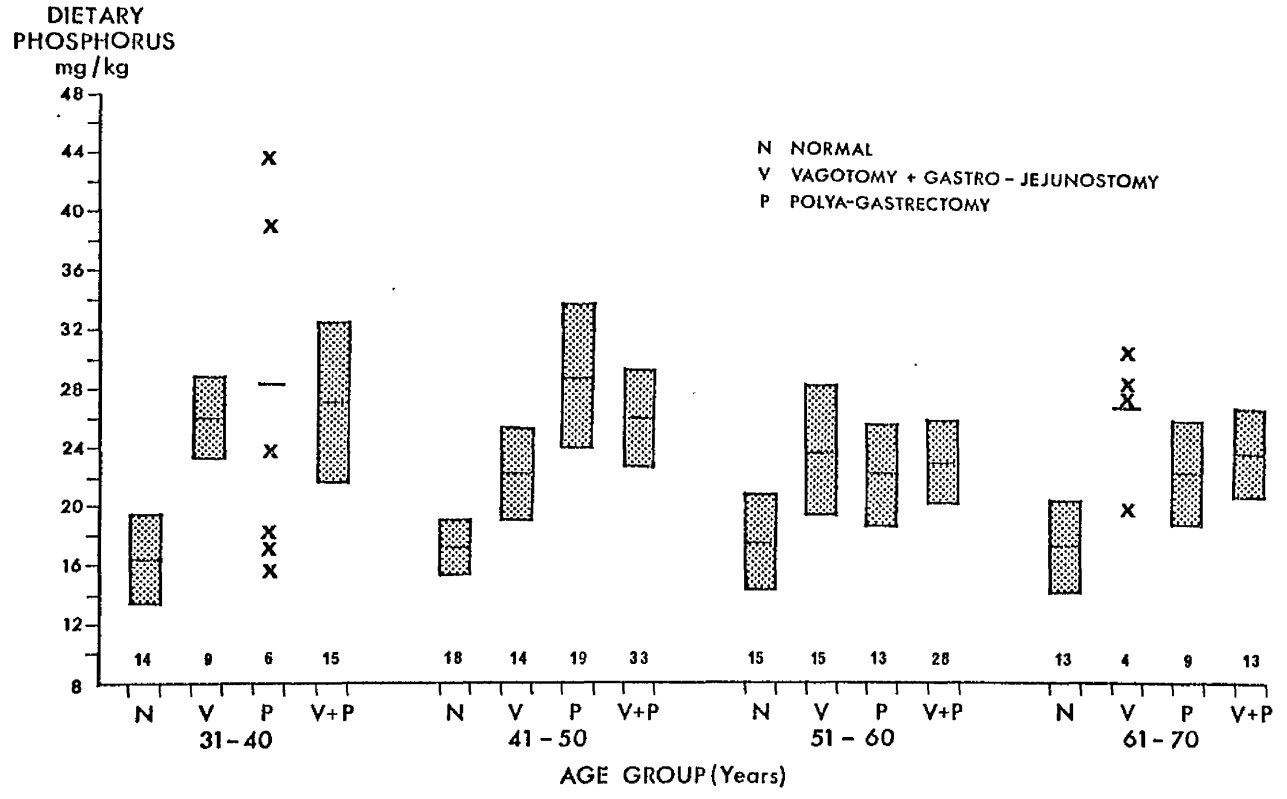
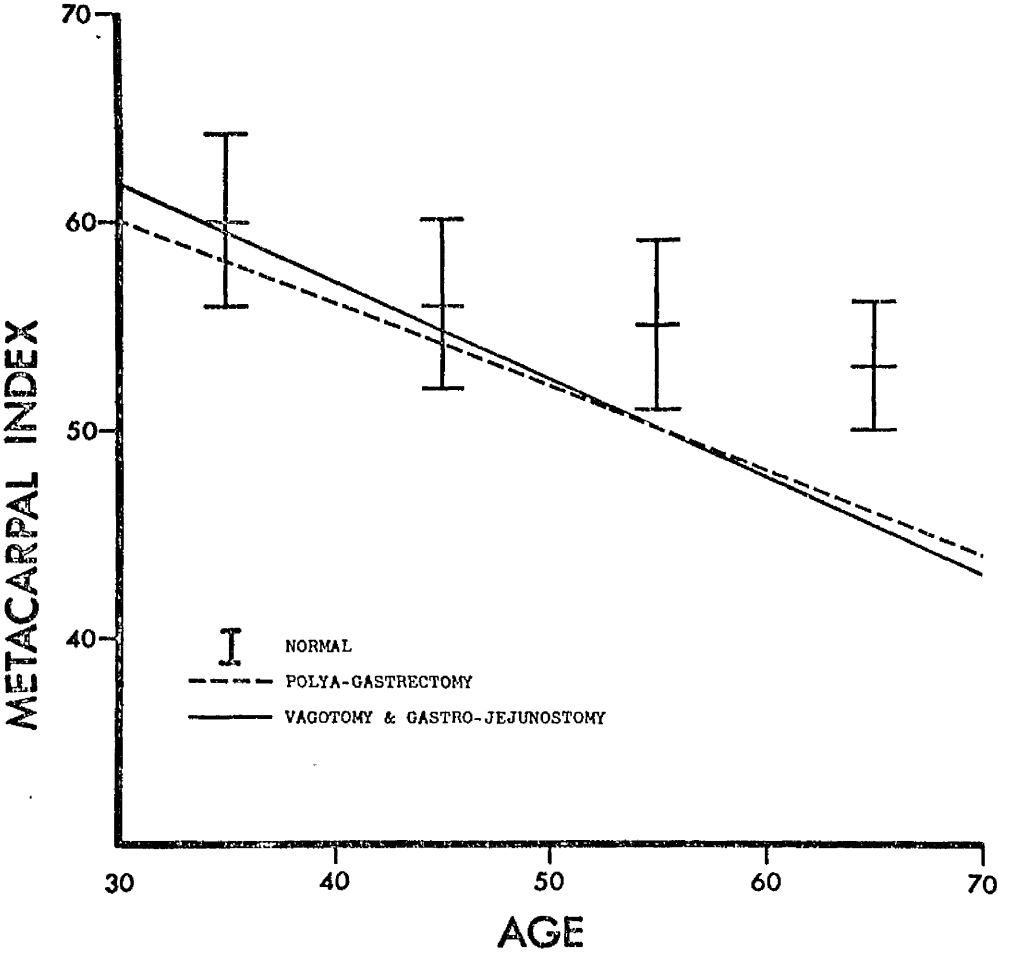


Fig 136 The dietary intake of phosphorus, divided by body weight, in patients who have undergone vagotomy with gastro-jejunostomy or Polya-gastrectomy, compared with normal male subjects. The mean and 2 S.E. range by decades are shown.



POLYA GASTRECTOMY	y=71 832- 0•395x (P<0•01)
VAGOTOMY & GASTROJEJUNOSTOMY	y=74 330- 0•430x (P<0•02)

Fig 137 The change in Metacarpal Index with age in the patients who have undergone vagotomy with gastro-jejunostomy or Polya-gastrectomy, compared with normal male subjects. The mean and 2 S.E. range for the normal subjects are shown as they show no significant fall with age (see Table LI).

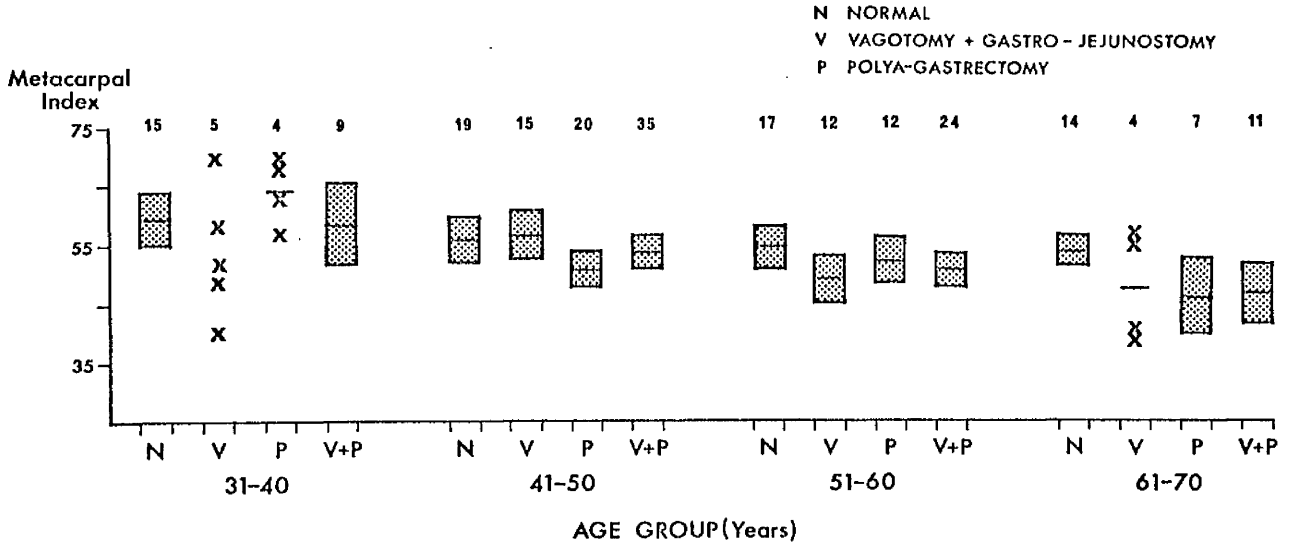


Fig 138 The change in Metacarpal Index with age, by decades, in patients with vagotomy and gastro-jejunostomy or Polya-gastrectomy, compared with normal subjects. The mean and 2 S.E. range are shown (see Table LII).

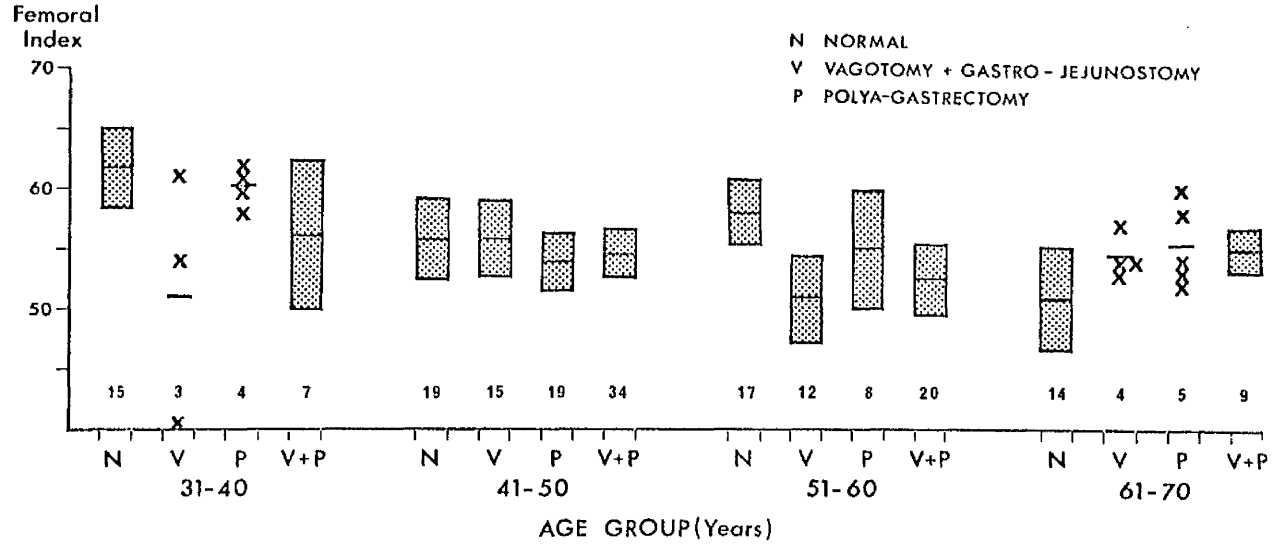


Fig 139 The relation between Femoral Index and age in patients with vagotomy and gastro-jejunostomy or Polya-gastrectomy, compared with normal subjects. The mean and 2 S.E. range by decades are shown (see Table LII).

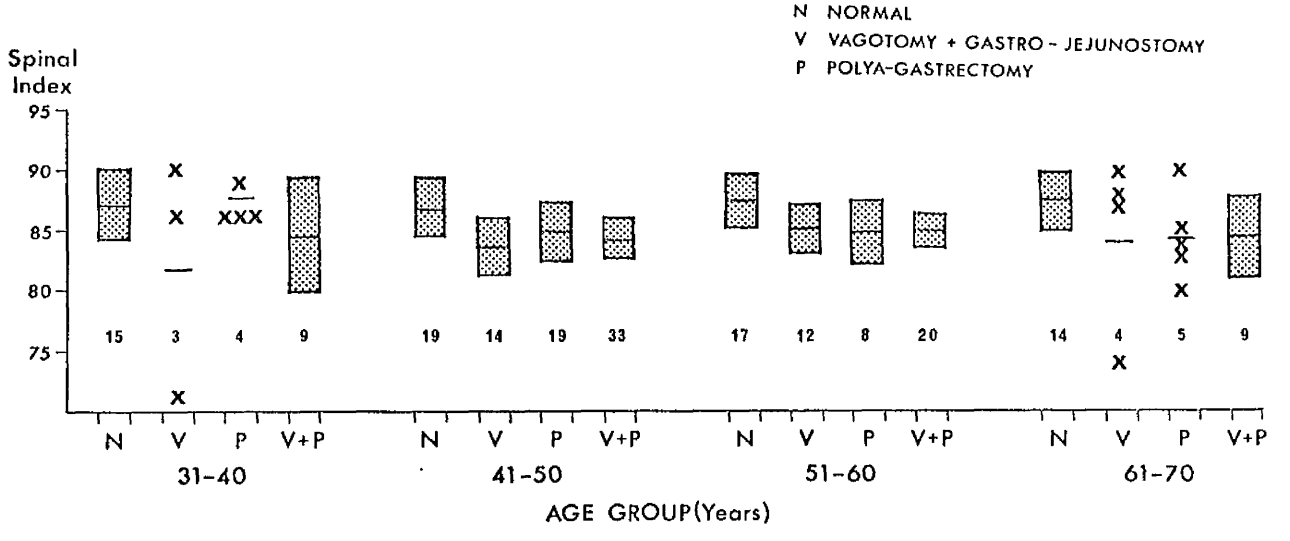


Fig 140 The relation between Spinal Index and age in patients with vagotomy and gastro-jejunostomy or Polya-gastrectomy, compared with normal subjects. The mean and 2 S.E. range by decades are shown (see Table LII).

CHAPTER IX

	Gradient	Intercept	r	P
S.A.E. percentile v time on therapy	- 0.075	35.73	0.039	n.s.
T.C.M. percentile v time on therapy	- 0.039	32.00	0.020	n.s.
Change in S.A.E. percentile v time on therapy	- 0.181	0.81	0.158	< 0.01
Change in T.C.M. percentile v time on therapy	- 0.122	0.84	0.150	< 0.01

TABLE LIII

The relation between the percentile value and the change in the percentile value of the S.A.E. and the T.C.M., and the time on therapy of 94 patients treated for 18 to 54 months with calcium supplements.

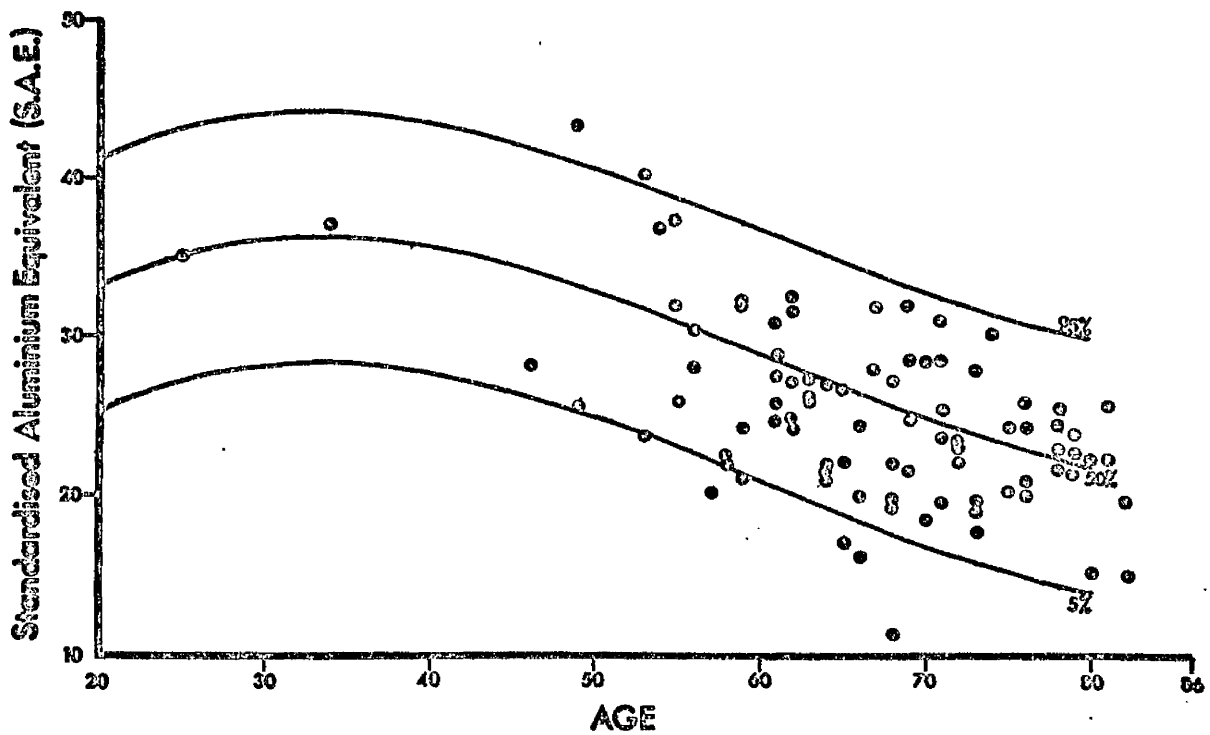


Fig 141 The relation between the initial whole bone density (S.A.E.) and age in female patients thought to be osteoporotic, compared with the normal range. The 5 and 95 percentile values for the normal subjects are shown.

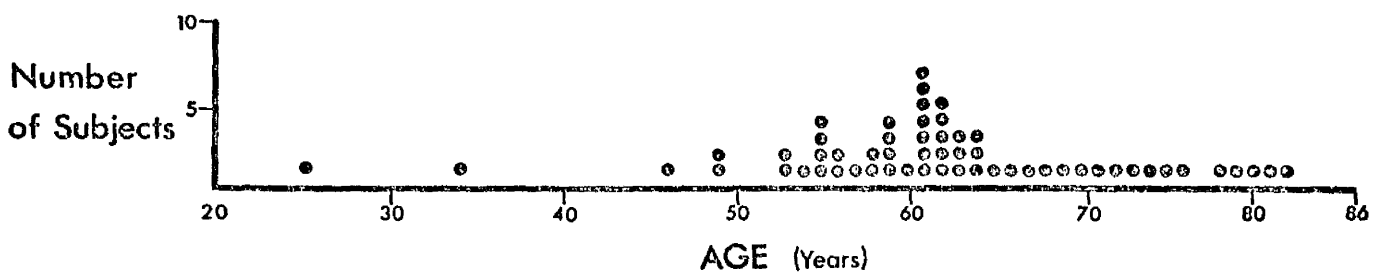


Fig 142 The age distribution of the female patients treated with calcium supplements.

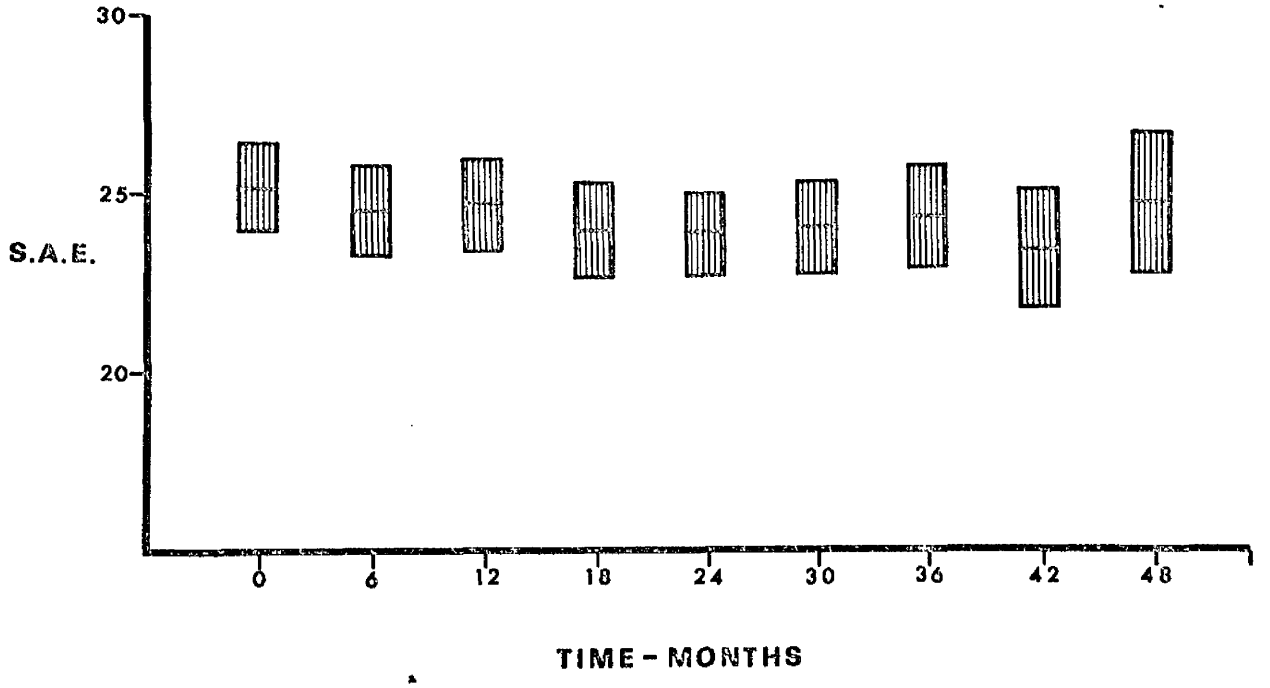


Fig 143 The whole bone density (S.A.E.) plotted against the time since starting therapy in 6-monthly intervals. The mean and 2 S.E. range are shown.

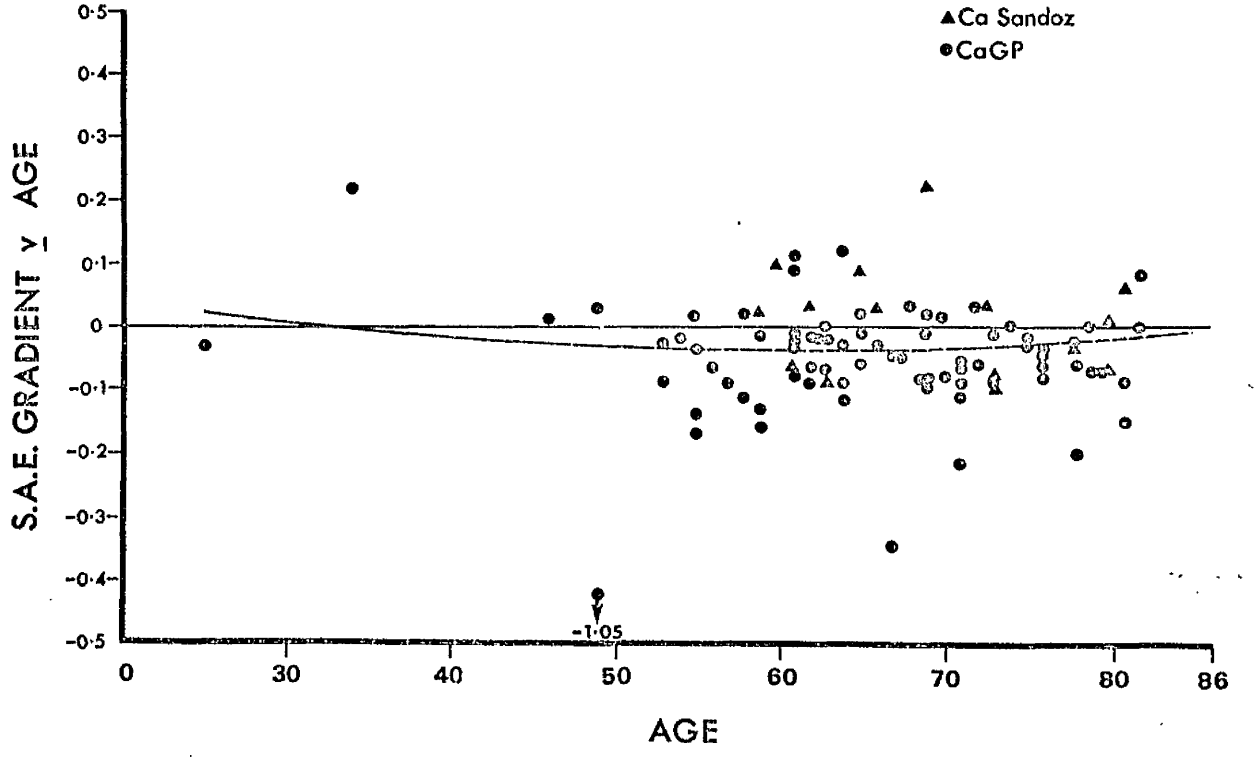


Fig 144 The gradient of the change with time in the S.A.E. in patients treated with calcium supplements, compared with the rate of change in S.A.E. in the normal population with time.

▲ ● Rate of change of S.A.E. in treated patients.

– – – Rate of change of S.A.E. in normal subjects.

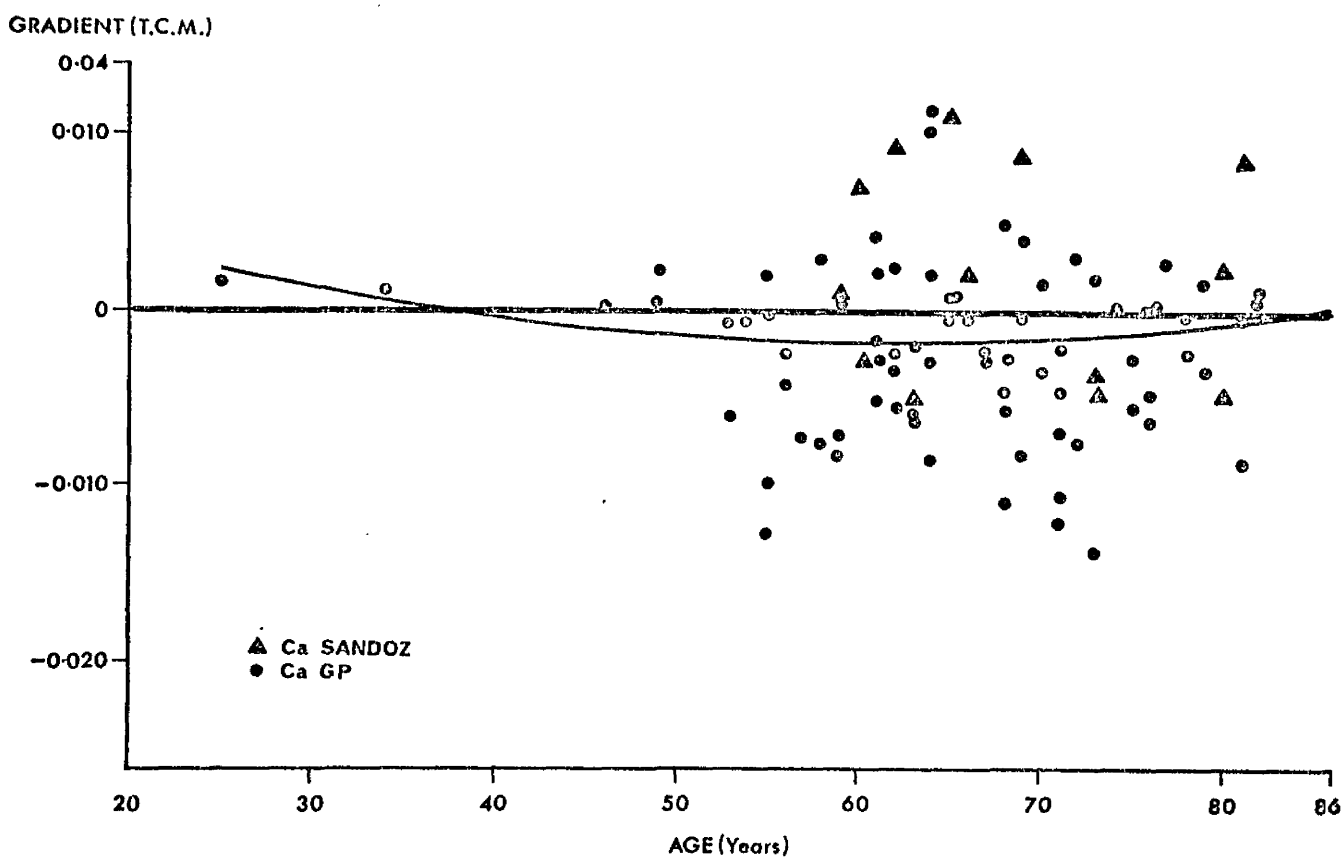


Fig 145 The gradient of the change with time of the T.C.M. (Total Cortical Mineral per unit length of metacarpal) in patients treated with calcium supplements, compared with the rate of change in T.C.M. in the normal subjects with time.

▲ ● Rate of change of T.C.M. in treated patients.

Rate of change of T.C.M. in normal subjects.

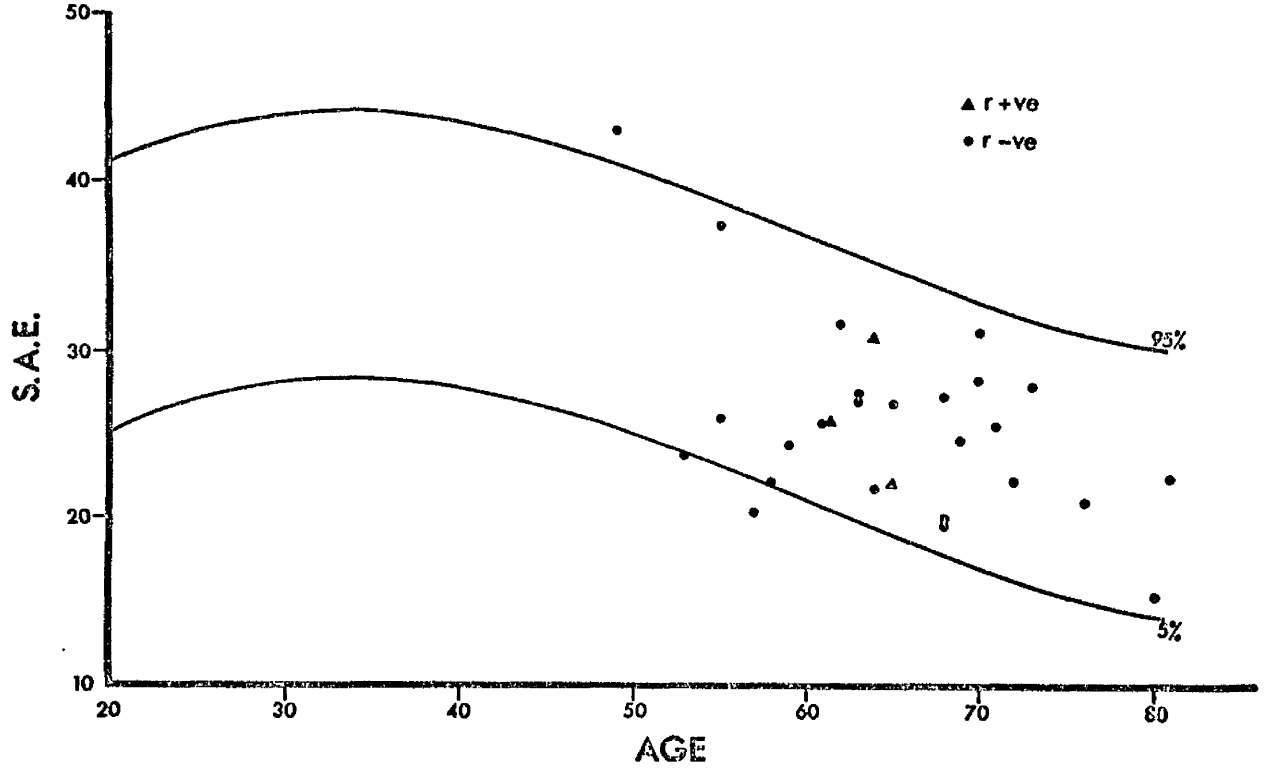


Fig 146 The initial whole bone density (S.A.E.) in patients who showed a significant increase or decrease in S.A.E. during the period of calcium therapy, plotted against age, compared with the normal population. The 5 and 95 percentile limits for the normal population are shown.

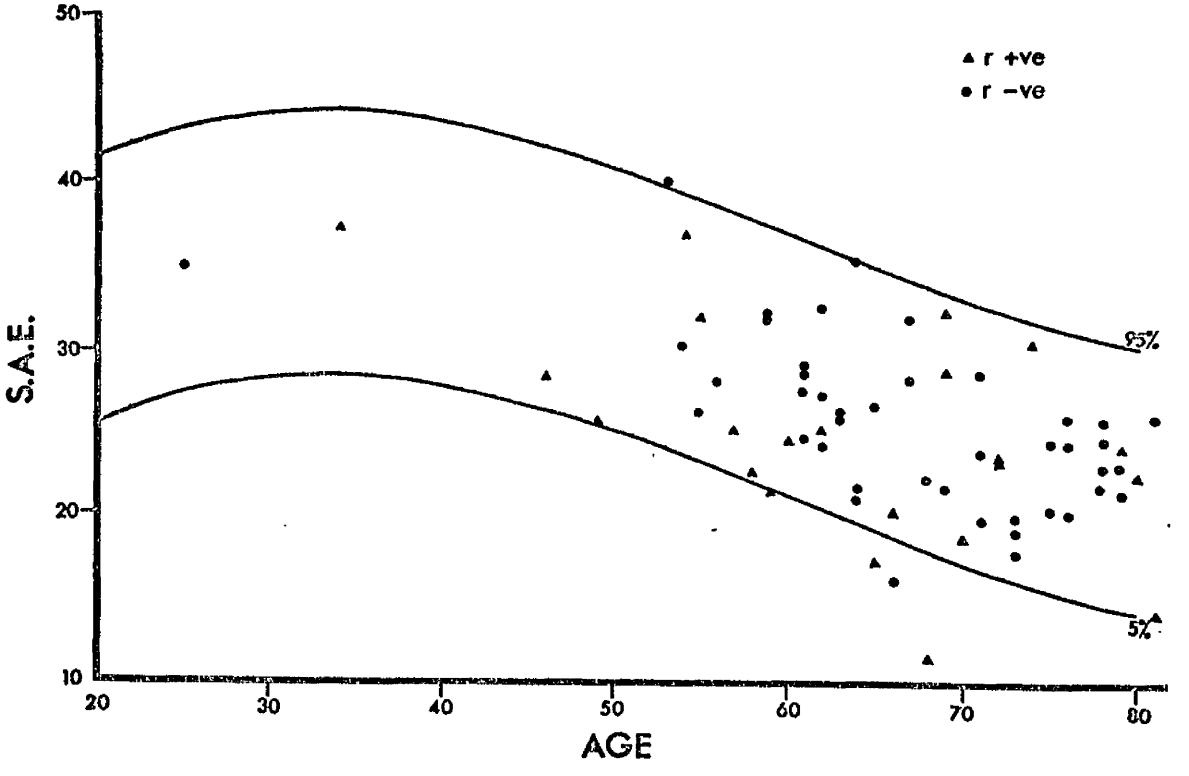


Fig 147 The initial whole bone density (S.A.E.) in those patients who showed no significant change in S.A.E. while on calcium therapy, plotted against age, compared with the normal population. The 5 and 95 percentile limits for the normal population are shown.

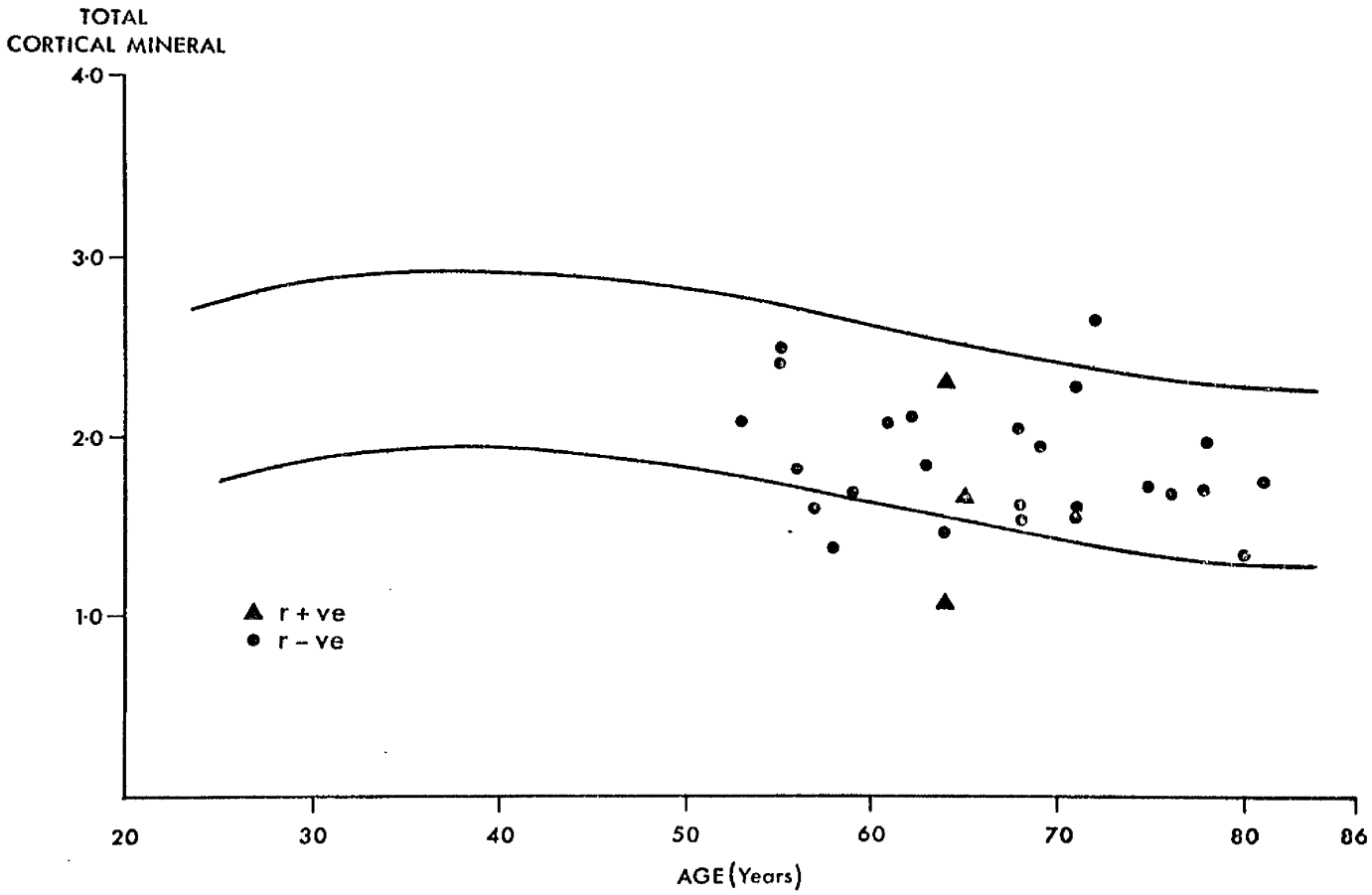


Fig 148 The initial T.C.M. in those patients who showed a significant increase or decrease in T.C.M. during the period of calcium therapy, plotted against age, compared with the normal population. The 5 and 95 percentile limits for the normal population are shown.

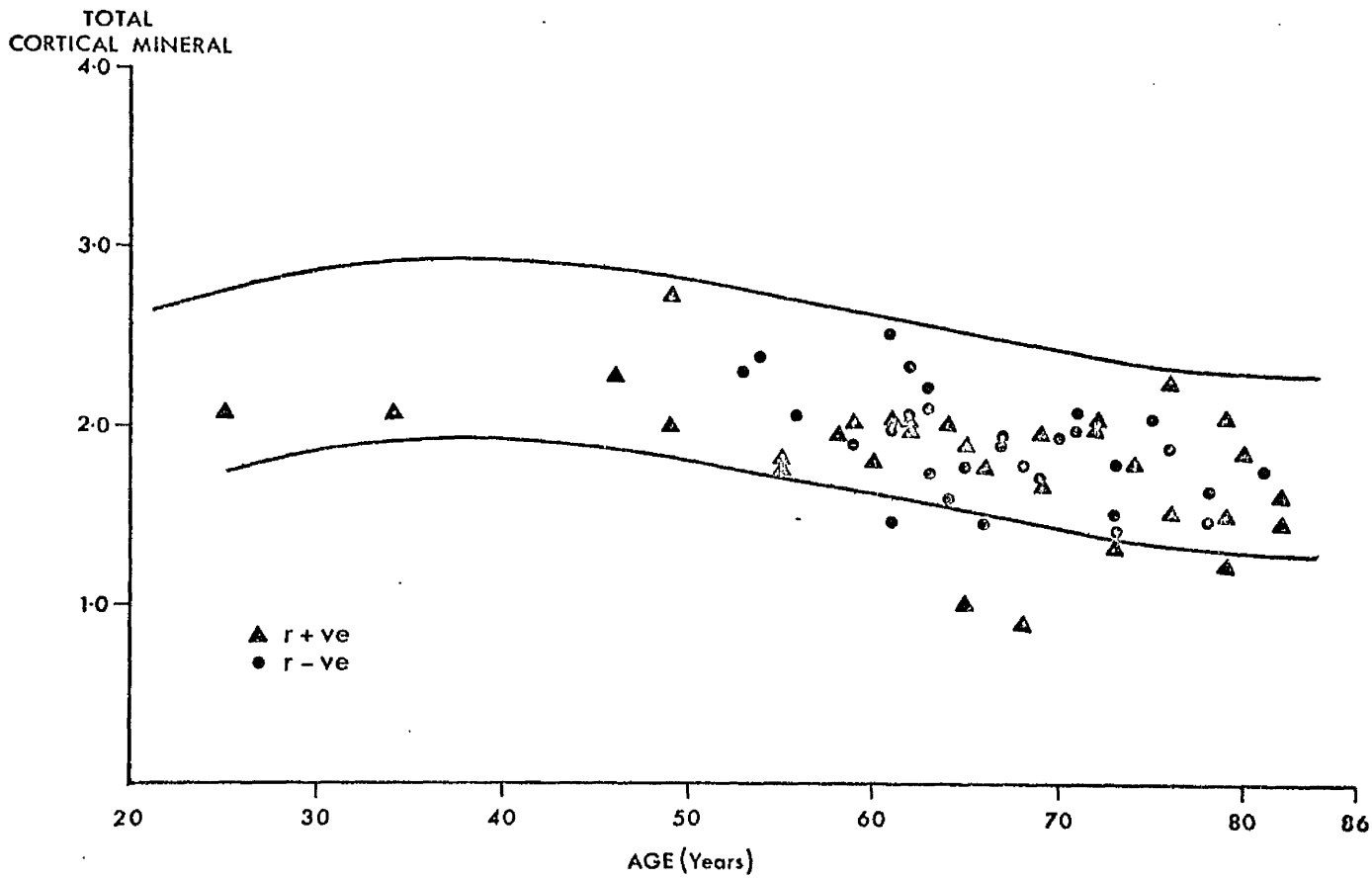


Fig 149 The initial T.C.M. in those patients who showed no significant change in T.C.M. while on calcium therapy, plotted against age, compared with the normal population. The 5 and 95 percentile limits for the normal population are shown.

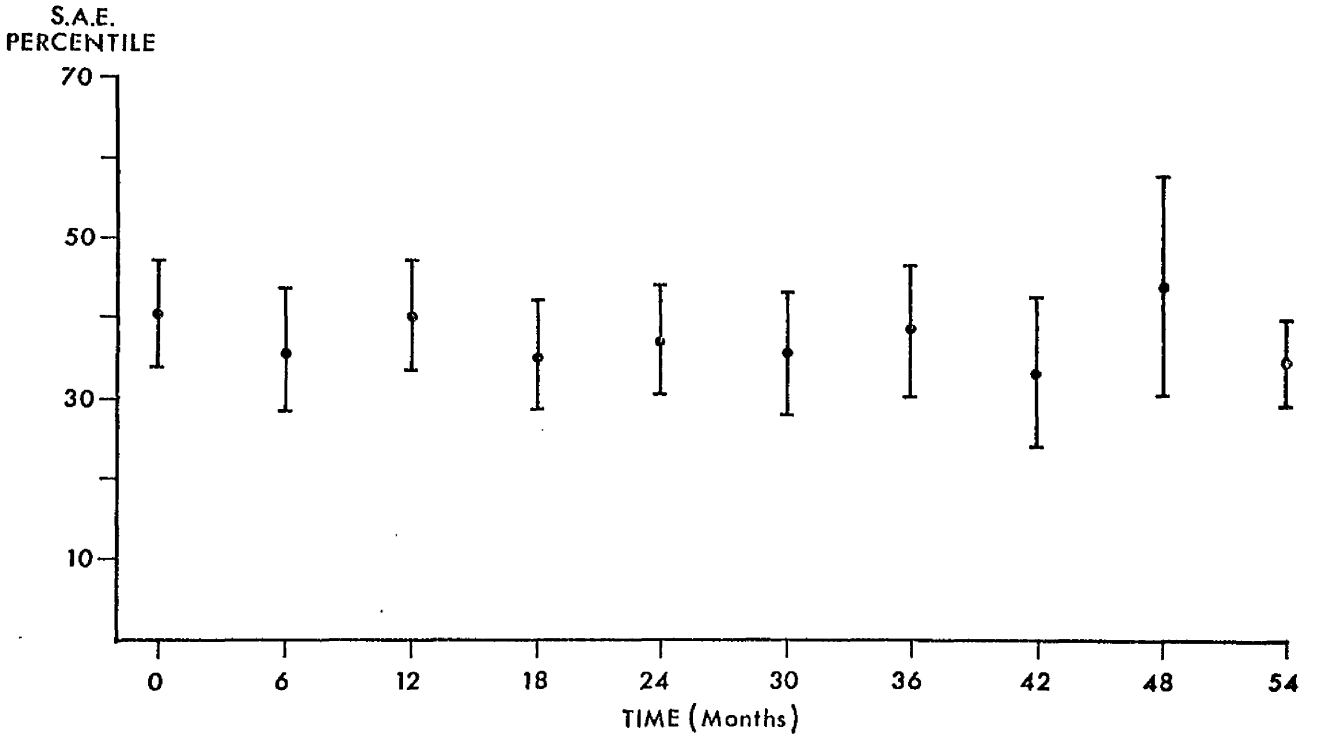


Fig 150 The change in percentile value of S.A.E. with time since starting calcium therapy. The mean and 2 S.E. range are shown.

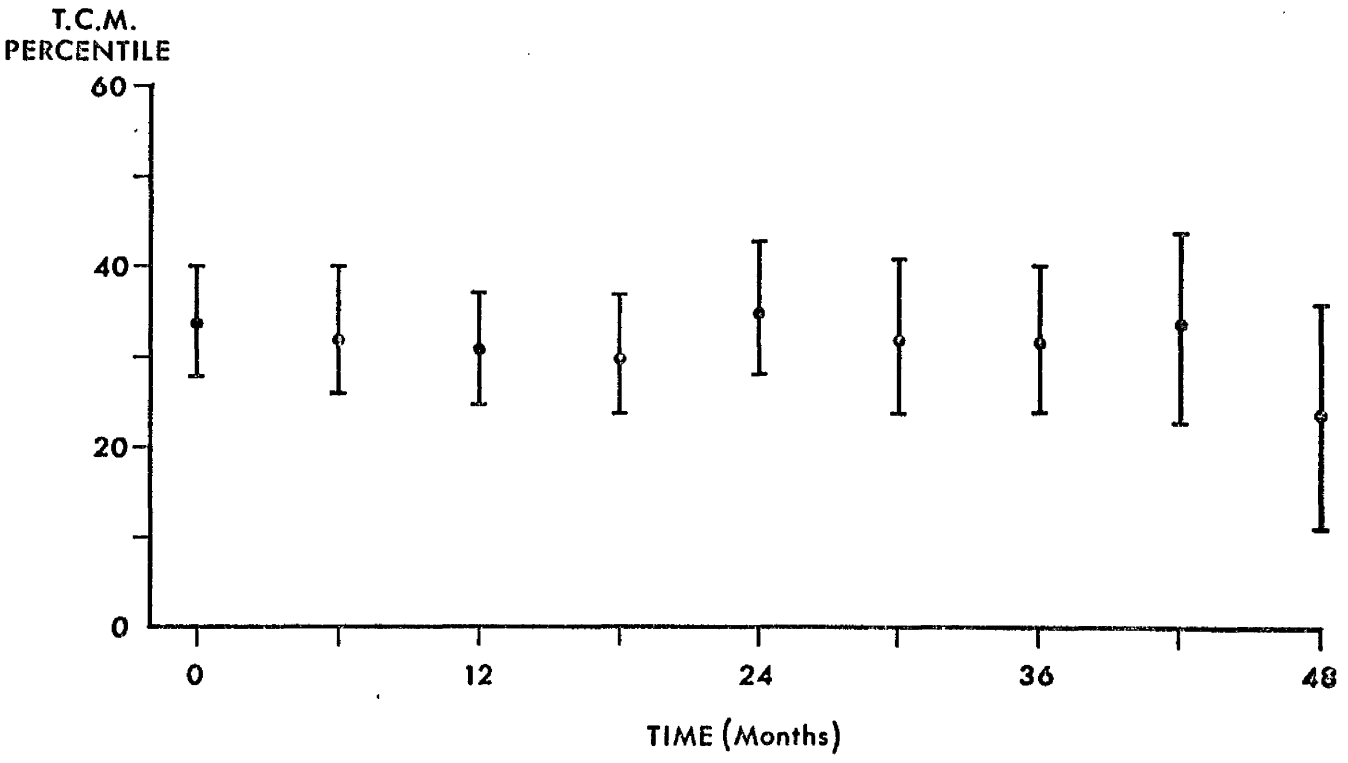


Fig 151 The change in percentile value of T.C.M. with time since starting calcium therapy. The mean and 2 S.E. range are shown.

CHAPTER X

Subject		Sex	Age	Diagnosis	S.A.E.	S.A.E. Percentile	Calcium Intake (gm/day)			Fat Intake (g/day)	
							A	B	C	A & B	C
1	M.S.	F	64	Osteoporosis	23.1	26	0.233	1.373	1.473	41.26	41.56
2	H.G.	F	73	Osteoporosis	20.9	31	0.267	1.407	1.507	36.03	36.33
3	T.R.	F	65	Osteoporosis	19.2	9	0.327	1.467	1.567	44.00	43.30
4	A.P.	F	70	Osteoporosis	20.5	24	0.234	1.383	1.483	32.87	33.17
5	M.K.	F	69	Osteoporosis	23.5	41	0.349	1.489	1.589	42.66	42.96
6	A.C.	M	61	Cerebrovascular accident	27.3	22	0.733	1.837	-	65.90	-

TABLE LIV

A Period of basic intake

B Calcium supplements added to give 1.14 gm additional calcium. (calcium glycerophosphate 2 gm t.i.d. to subjects 1-5 and calcium Sandoz tabs. 1, t.i.d. to subject 6).

C Substitution of calcium glycerophosphate for dried skimmed milk (1.24 gm calcium daily supplementary to basic intake in patients 1 - 5.

Subject	Sex	Age	CALCIUM BALANCE A	CALCIUM BALANCE B	CALCIUM BALANCE C	URINARY HYDROXYPROLINE A	URINARY HYDROXYPROLINE B	URINARY HYDROXYPROLINE C
M.S.	F	64	-3.23	-2.00	+4.29	0.555	0.518	0.386
H.G.	F	73	-1.64	-1.70	+2.18	0.380	0.407	0.425
J.R.	F	65	-2.57	+1.47	+2.17	0.815	0.677	0.688
A.P.	F	70	-3.42	-2.00	+0.60	0.412	0.499	0.382
M.M.	F	69	-0.73	+1.13	+3.65	0.524	0.484	0.539
Mean			-2.32	-0.62	+2.58	0.537	0.517	0.485

TABLE LV

Calcium balance and urinary hydroxyproline excretion in 5 osteoporotic subjects.

PERIOD A Basic intake

PERIOD B Basic intake plus calcium glycerophosphate (1.14 gm supplementary calcium)

PERIOD C Basic intake plus dried skimmed milk (1.24 gm supplementary calcium).

Subject	Faecal dry weight g/24hr			Faecal acid fat g/24hr			Total faecal fat g/24hr			Faecal calcium mg/24hr		
	A	B	C	A	B	C	A	B	C	A	B	C
1	15.5	21.2	28.4	1.55	3.90	4.29	2.36	4.36	4.70	197.0	1201.8	2063.8
2	14.1	17.3	19.2	1.17	1.80	2.16	1.48	2.00	2.50	237.4	1180.0	1084.0
3	18.4	20.4	20.5	1.08	1.64	1.57	1.65	2.11	2.20	217.3	1160.0	1083.3
4	14.0	20.8	17.8	2.25	4.02	2.54	4.41	7.44	3.79	213.2	1136.8	1150.3
5	13.6	19.8	22.3	0.79	1.29	1.27	1.30	1.99	2.05	260.6	1182.0	1286.3
6	17.1	19.6	-	1.91	3.04	-	2.67	4.22	-	-	-	-
Mean	15.45	19.85***	21.64*	1.46	2.62**	2.37	2.31	3.69*	3.05	225.1	1172.1***	1333.5***
S.D.	±1.94	±1.39	±4.13	±0.55	±1.20	±0.53	±1.15	±2.15	±1.14	±24.5	±24.7	±416.5

Significant difference from pretreatment level: *p<0.05, **p<0.02, ***p<0.01

TABLE LVI

Mean weight of faecal dry weight, faecal fat and faecal calcium before (A) and after increase in calcium content of diet by 1.14g/day of calcium glycerophosphate (B) and 1.24g/day of calcium as 1½ pints of skimmed milk (C)

	Deoxycholic Acid mg./24hr			Lithocholic Acid mg./24hr			Total mg./24hr		
	A	B	C	A	B	C	A	B	C
1	42.0	85.6	97.1	44.0	56.7	47.3	86.0	142.3	144.4
2	27.7	43.4	26.0	18.6	37.3	23.2	46.3	80.7	49.2
3	25.8	36.5	29.9	8.9	30.8	26.5	34.7	67.3	56.4
4	8.1	15.3	27.1	8.8	10.8	19.8	16.9	26.1	46.9
5	66.6	74.5	92.7	12.5	43.4	53.3	79.1	117.9	146.0
6	22.4	31.1	-	21.9	21.4	-	44.3	52.5	-
Mean	32.10	47.73*	54.56	19.12	33.40*	34.02	51.22	81.13**	88.52*
S.D.	±20.09	±26.92	±36.89	±13.28	±16.25	±15.20	±26.49	±42.74	±51.73

*p<0.05
**p<0.02 Significant difference from pretreatment level

TABLE LVII

Mean faecal bile acid output (mg./24hr) before (A) and after increase in calcium content of diet by 1.14g./day of calcium as calcium glycerophosphate (B) and 1.24g./day of calcium as $1\frac{1}{2}$ pints of skimmed milk (C)

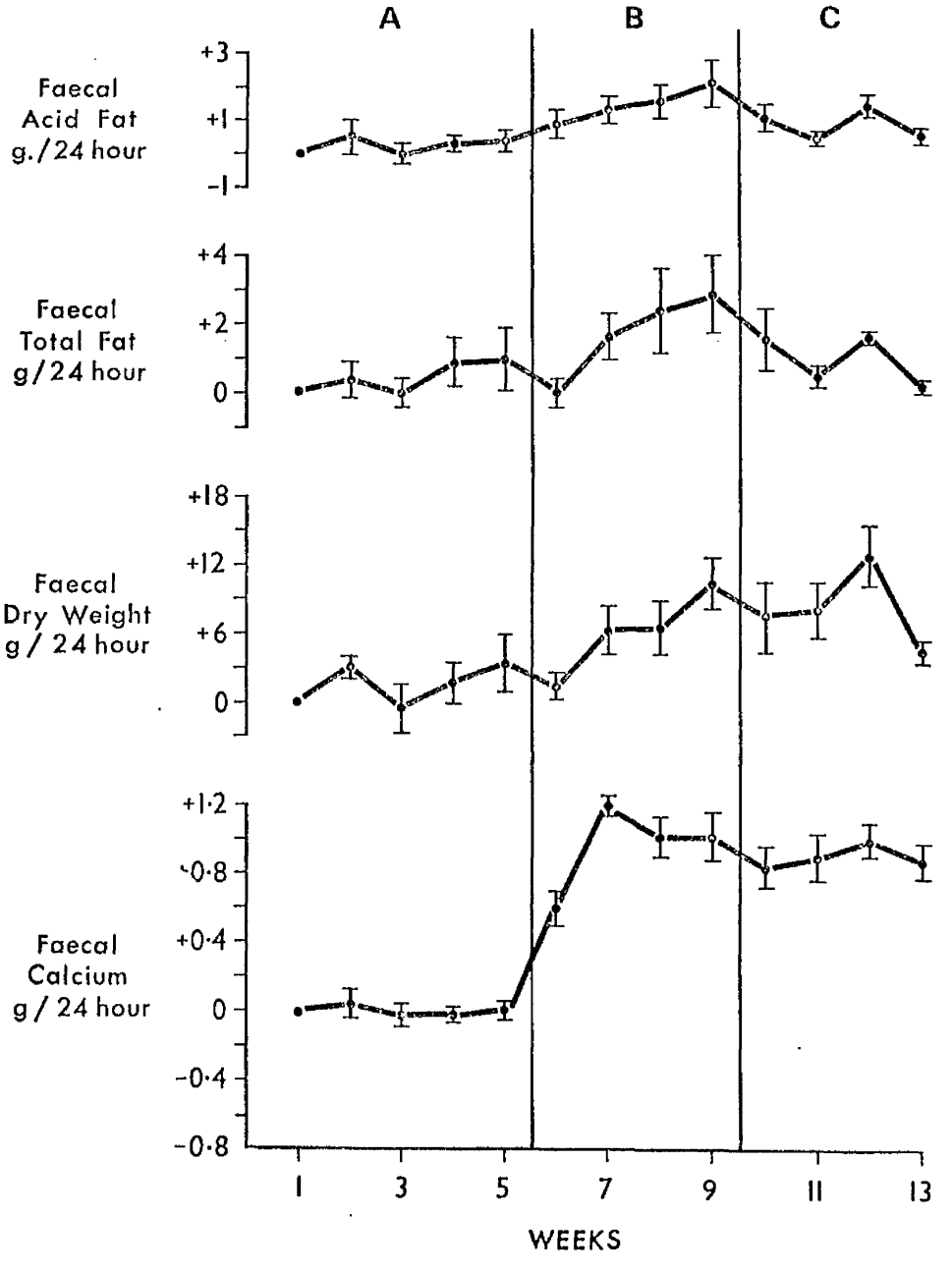


Fig 152 The mean weekly changes ($\pm$ 1 S.E.) in faecal bile acid excretion from the first week of the basal period.

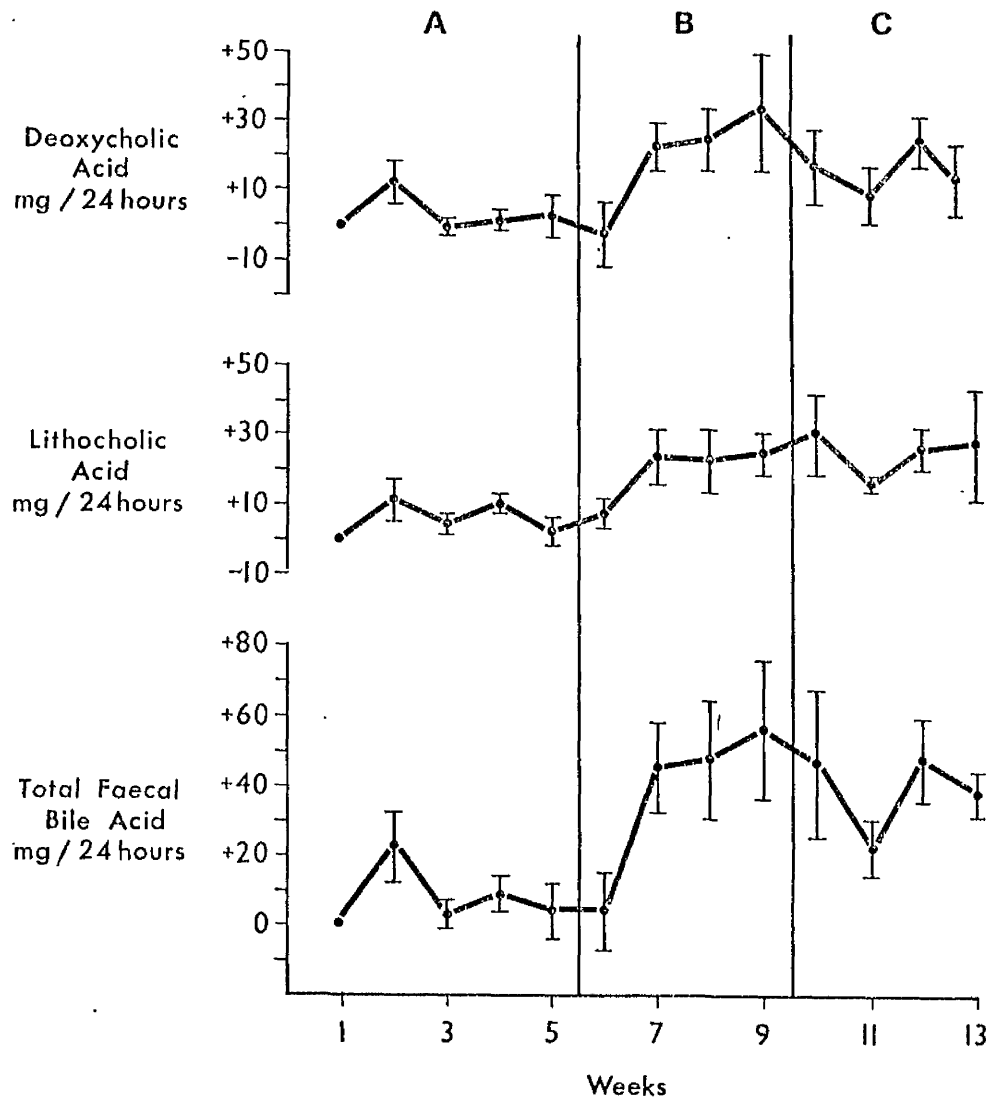


Fig 153 The mean weekly changes ($\pm$ 1 S.E.) in faecal acid fat, total fat, dry weight and calcium from the first week of the basal period.